Landfill Leachate as a Source of Plant Nutrients

CHENG Chung-yin

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Philosophy
in
Biology

Abstract

Landfill leachate has long been recognized as a potential source of water pollutants and must therefore be treated before disposal. It is characterized by high level of salts and ammoniacal-nitrogen (NHx-N) and high organic loading. Irrigation with landfill leachate provides a means of wastewater recycling as well as nutrient reuse. However, experimental studies have come up with both positive and detrimental effects of landfill leachate. Research on leachate irrigation mainly focused on maximizing treatment efficiency. Important parameters of leachate application, such as dilution level, were seldom considered in terms of phytotoxicity, and this resulted in contradictory outcomes.

Landfill leachate contains considerable amount of NHx and other nutrients which can be assimilated for plant growth. The value of leachate on plants applied through irrigation was examined. An attempt was made to use phytotoxicity data for the design of a leachate irrigation plan to safeguard the recipient plants from growth inhibition or death.

Leachates from landfills of different ages were examined for their phytotoxicity by seed germination/root elongation tests using seeds of *Brassica chinensis* (Chinese white cabbage) and *Lolium perenne* (perennial ryegrass). Their EC50s ranged from 4% to 30% v/v, which varied remarkably with the age of landfill. Seedlings of 12 tree species were grown in pots, which were irrigated with landfill leachate at the EC50 levels (with tap water as control). No tree mortality or growth inhibition was observed in 90 days of leachate application. Chlorophyll fluorescence measurement also showed

that plants receiving leachate did not suffer from reduction in photosynthetic efficiency. *Litsea glutinosa* (pond spice) and *Hibiscus tiliaceus* (sea hibiscus) had remarkable growth, and other non N-fixers were not inferior to the N-fixing *Acacia auriculiformis* (earleaf acacia). Moreover, leachate irrigation improved soil N content, though P deficiency is a problem. The seed germination test provided a conservative estimate of the phytotoxicity of landfill leachate. Plants irrigated can be protected from growth inhibition when the leachate irrigation plan is designed in light of phytotoxicity data.

An N balance study was conducted with a soil column design to investigate the fate and behavior of leachate N in soil, plants and soil percolate. Soil-plant systems with leachate irrigation were compared with those receiving application of mineral fertilizer (Nitrophoska 15:15:15). The plant growth in the leachate and fertilizer treatments did not differ significantly in most species tested. Their growth performances again showed that phytotoxicity tests using germinating seeds could suggest a safe upper limit of the application rate.

The results also suggest the importance of plants in retaining nutrients. Gain in the N capital was only observed in the treatment groups with vegetative cover. Leachate irrigation for 12 weeks brought about 250 - 1050 kg N ha^{-1} stored in the biomass and soil, depending on the type of vegetation. Without vegetative cover, a substantial amount of N was lost in the soil percolate. The N added with leachate was insufficient to compensate for the leaching loss. The large leaching loss suggested that the application rates being tested were excessive compared with plant requirement. If the aim of leachate irrigation is solely for nutrient supplement, there is still room for reducing the application rate of leachate without compromising the nutrient needs.

摘要

堆填區滲濾污水(滲濾液)是一種成分複雜的高濃度有機廢水，倘不加處理而直接排放，其高濃度的氨氮及生物需氧量有可能構成嚴重的污染，因此適當的處理尤爲重要。其中土地處理法(或土壤灌溉法)是最早採用的一種污水處理方式，即在人爲調控的前題條件下，把污水投配到土地上，其在處理生活污水上已具相當成熟的運作管理經驗。利用土地作爲污水處理的媒體，不但可淨化水質，亦能爲植被提供水份及養份。然而有關堆填區滲濾污水對植被的影響則未有明確定論。有研究發現灌溉滲濾液能加快植物的生長，亦有報告指高濃度的滲濾液對植被構成危害。

堆填區滲濾液有別於一般城市污水，其成份及毒性會隨堆填區的年份、氣候及廢物成份而有所不同。然而大部份的研究集中於優化土地及植物在淨化堆填區滲濾液的效能。在設計灌溉實驗時往往忽略個別滲濾液樣本的成份及植物毒性，而導致實驗間的結果有異。本研究旨在探討堆填區滲濾液的應用價值；利用簡單的植物毒性檢測方法評估個別滲濾污水樣本的毒性，繼而結合毒性檢測數據於利用滲濾液灌溉的規劃中，以保障植被免受損害。

首部份研究利用小白菜(*Brassica chinensis*)及多年生黑麥草(*Lolium perenne*)的種子萌芽測試評估不同年份堆填區的滲濾液之植物毒性。其半數有效濃度（EC50）爲 4% 至 30% 不等。一般而言，滲濾液之濃度及植物毒性均隨堆填區關閉年期遞減。

第二部份的實驗以稀釋至半數有效濃度(EC50)(以種子萌芽測試爲基礎)的滲濾液灌溉多個原生及引進的樹木品種，以測試它們對滲濾液的不同反應。在 90 天的灌溉期內並未發現死株，而滲濾液亦沒有抑制樹苗的生長。植物健康指標(如 葉綠素螢光 chlorophyll fluorescence) 顯示所有樹苗的光合作用效能並沒有受滲濾液所影響。在受測試的 12 個樹木品種中，潺槁(*Litsa glutinosa*)及黃槿(*Hibiscus tiliaceus*)在滲濾液灌溉下迅速生長，其生長率較固氮植物耳果相思(*Acacia auriculiformis*)爲高。另一方

面，經滲濾液灌溉的土壤，其氨氮及硝酸氮含量顯著增加，然而磷含量卻仍然偏低。結果顯示種子萌芽測試對滲濾污水的毒性較爲敏感，它能就個別滲濾污水樣本的毒性作保守可靠的評估。

接續的氮平衡(或稱氮收支)實驗分析在土壤上投配/灌溉滲濾液後，當中的氮化合物在泥土、植物及土壤水份中的分佈及轉化情況。植物分別接受滲濾液灌溉或施用化學肥料(Nitrophoska 15:15:15)後，其生長率並沒有顯著差異。顯示植物能有效地利用來自化學肥料及稀釋堆填區滲濾液的養份。然而，在所有施用滲濾液的實驗組中，均錄得大量的氮隨土壤滲漏流失，顯示氮施用量遠超過植被所需。倘灌溉堆填區滲濾液旨在爲植物提供養份，其施用量仍有相當的下調空間。另一方面植被在保持土壤/植物系統中的養份扮演著重要的角色。施用滲濾液於有植被的土壤後，有相當於 250 - 1050 kg ha^{-1} 的氮被保留於土壤/植物系統中，其氮儲備的增幅因應植被種類而有所不同。相比之下，於沒有植被的實驗組上，雖然施用滲濾液能增加其土壤中的氨氮及硝酸氮含量，惟增幅並不足以抵銷隨土壤滲漏的流失。植物透過蒸騰作用減少了土壤滲漏，並攝取土壤中的氮轉化並保存於其生物量當中，從而減少流失。。總括而言，堆填區滲濾液能促進植物的生長及改善貧瘠土壤的氮含量，然而在灌溉用滲濾液時，必須因應其植物的毒性作適當稀釋。

Acknowledgements

I would like to express my cordial gratitude to my supervisor, Prof. L.M. Chu for his guidance and inexhaustible encouragement throughout my postgraduate study. Special gratitude is dedicated to Prof. K.C. Chau, Department of Geography and Resources Management, Prof. S.W. Chiu and Prof. P.K. Wong, Department of Biology, The Chinese University of Hong Kong and Prof. Ray McBride, Department of Land Resources Science, University of Guelph, for their knowledgeable advice.

I wish to acknowledge the Hui Hua Environmental Science and Technology Co. Ltd., Guangzhou, China and Pegasus Greenland Ltd., Hong Kong for their donations of plant materials. I am grateful to the Tai Tong Nursery of the Agriculture, Fisheries and Conservation Department, Hong Kong and the Flora Conservation Department of Kadoorie Farm and Botanic Garden for their provision of tree seedlings. Moreover, the study could not have been accomplished without the financial support of a UGC direct grant and the assistance from the Waste Facilities Business Unit of Environmental Protection Department and the Mine and Quarry Division of Civil Engineering Department, Hong Kong.

Many staff members in the Department of Biology have been very helpful. I am particularly grateful to Mr. Ben Yeung, Mr. Y.H. Yung and Dr. K.C. Chung for their skillful, invaluable suggestions and technical support. I wish to thank, Mr. Raymond Wong, Ms. Mimi Cheung, Mr. John C.W. Chong, Mr. Martin T.K. Tsui, Mr. Chiky C.Y. Wong, Ms. S.M. Ho, Mr. Mike C.C. Yu, Ms. Joyce K.W. Lau, Ms. Michelle M.S. Wong and Mr. M.K. Chung for their helpful advice and assistance. Also I am thankful to Mr. P.L. Chan, Ms. Christy L.H. Fu, Ms. Loreta L.L. Lee, Mr. Mike W.K. Leung, Ms. W.S. Li and Ms. M.W. Wong. Their experience is invaluable to the experimental design.

I am much obliged to Ms. Priscilla O.Y. Leung, Ms. H.Y. Chung, Ms. Vivian K.M. Pang, Ms. Annie O.N. Wong, and Ms. Sarah P.S. Yau for their sincere support in my postgraduate study.

I am also much indebted to the people who are not named here, but have helped me a lot in the past two years.

Table of contents

List of tables

List of figures

List of plates

Plant species used in the experiments

Species	Chinese name	Common name
Acacia auriculiformis	耳果相思	Earleaf acacia
Brassica chinensis	小白菜	Chinese white cabbage
Casuarina equisetifolia	木麻黃	Horsetail tree
Celtis sinensis	朴樹	Chinese hackberry
Cinnamomum camphora	樟樹	Camphor
Eucalyptus citriodora	檸檬桉	Lemon eucalyptus
Hibiscus tiliaceus	黃槿	Sea hibiscus
Liquidambar formosana	楓樹	Formosan sweetgum
Litsea glutinosa	潺槁	Pond spice
Lolium perenne	多年生黑麥草	Perennial ryegrass
Lophostemon confertus	紅膠木	Brush box
Melaleuca quinquenervia	白千層	Cajeput tree
Paspalum notatum	百喜草	Bahia grass
Sapium discolor	山烏桕	Mountain tallow tree/Tallow tree
Scolopia chinensis	刺柊	Chinese scolopia
Vetiveria zizanioides	香根草	Vetiver/Vetiver grass

Chapter 1 Introduction

1.1 Soil wastes as an environmental challenge

Wastes are a common problem of affluent societies. People who can afford greater convenience and more purchases tend to throw away more wastes. In the United States, the per-capita waste generation rate increased 68% in the past 20 years (Figure 1.1); about 160 million tonnes of wastes were disposed of in 2000 (USEPA 2002, 2003). Moreover, each year every person in European countries on average throws away 3.5 tonnes of solid wastes (European Communities, 2002). The amount of wastes generated in Europe increased by 10% between 1990 and 1995.

Hong Kong, a city with a service-based economy, generates less wastes than other countries with high industrial activities. However, the municipal wasteloads have increased in the past decades, mirroring the rapid expansion in the local economy and population over the same period (Figure 1.2). Dealing with the growing amount of wastes without damaging the environment has become a global issue in the new century.

1.2 Landfilling

Historically, health and safety have been the major concerns of waste management. Wastes are managed in a way that minimizes the risk to human health. Sanitary landfilling is probably the most common method of waste disposal. As early as the 1970s, nearly 80% of the solid wastes generated in US cities were buried (Baum and Parker, 1974).

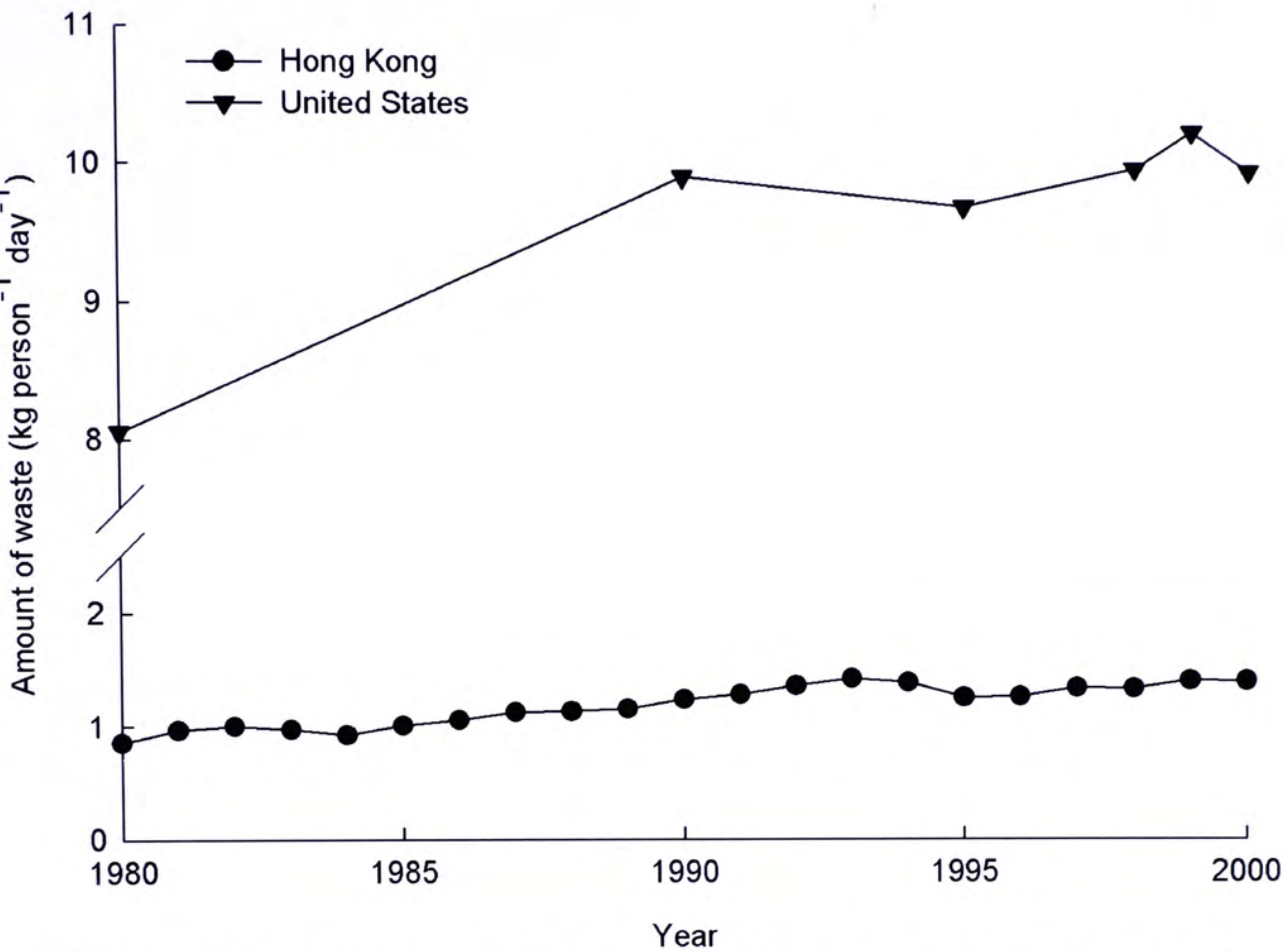


Figure 1.1 Per-capita waste generation in United States and Hong Kong (EPD, 2001; USEPA 2002, 2003).

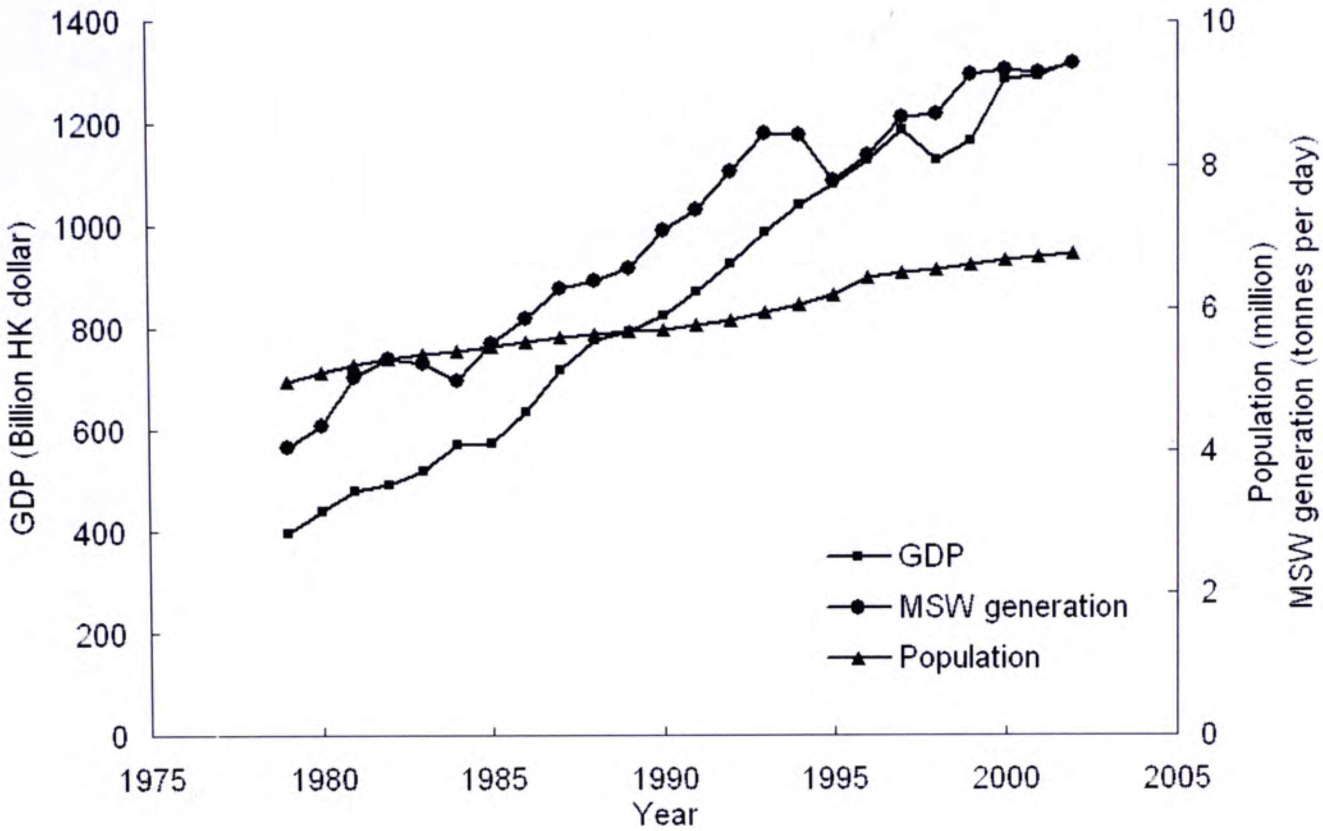


Figure 1.2 The gross domestic product (GDP), population and municipal solid wastes arising in Hong Kong (EPD, 2001; CSD, 2003).

Solid waste disposal in landfills remains the most economic form of disposal in the vast majority of cases (Carra and Cossu, 1990). In the late 1990s, depending on the location, up to 95% of solid wastes generated worldwide were disposed of in landfills (Cossu, 1989). It is anticipated in the future, that landfills will continue to be the most attractive disposal method for solid wastes, despite greater emphasis in waste reduction, reuse and recycling.

Effective waste management needs to look at its effect on the environment. Landfilling has been defined as "*the engineered deposit of wastes onto and into land in such a way that pollution or harm to the environment is prevented and, through restoration, land provided may be used for another purpose*" (Westlake, 1995). An effective landfill design, control of waste degradation by-products and post-closure management are essential for minimizing the environmental nuisances.

1.2.1 Waste degradation

It is well known that a landfill could be viewed as a large-scale bioreactor as wastes can be broken down into simpler compounds by aerobic and anaerobic microorganisms, leading to the formation of gas and leachate. The degradation process consists of several interdependent stages: aerobic phase, anaerobic acetogenic phase and anaerobic methanogenic phase (Figure 1.3) (Pfeffer, 1992; Tchobanoglous *et al.,* 1993).

When municipal solid wastes (MSW) is deposited within the landfill, oxygen entrapped within the void spaces is rapidly depleted as a result of microbial activity.

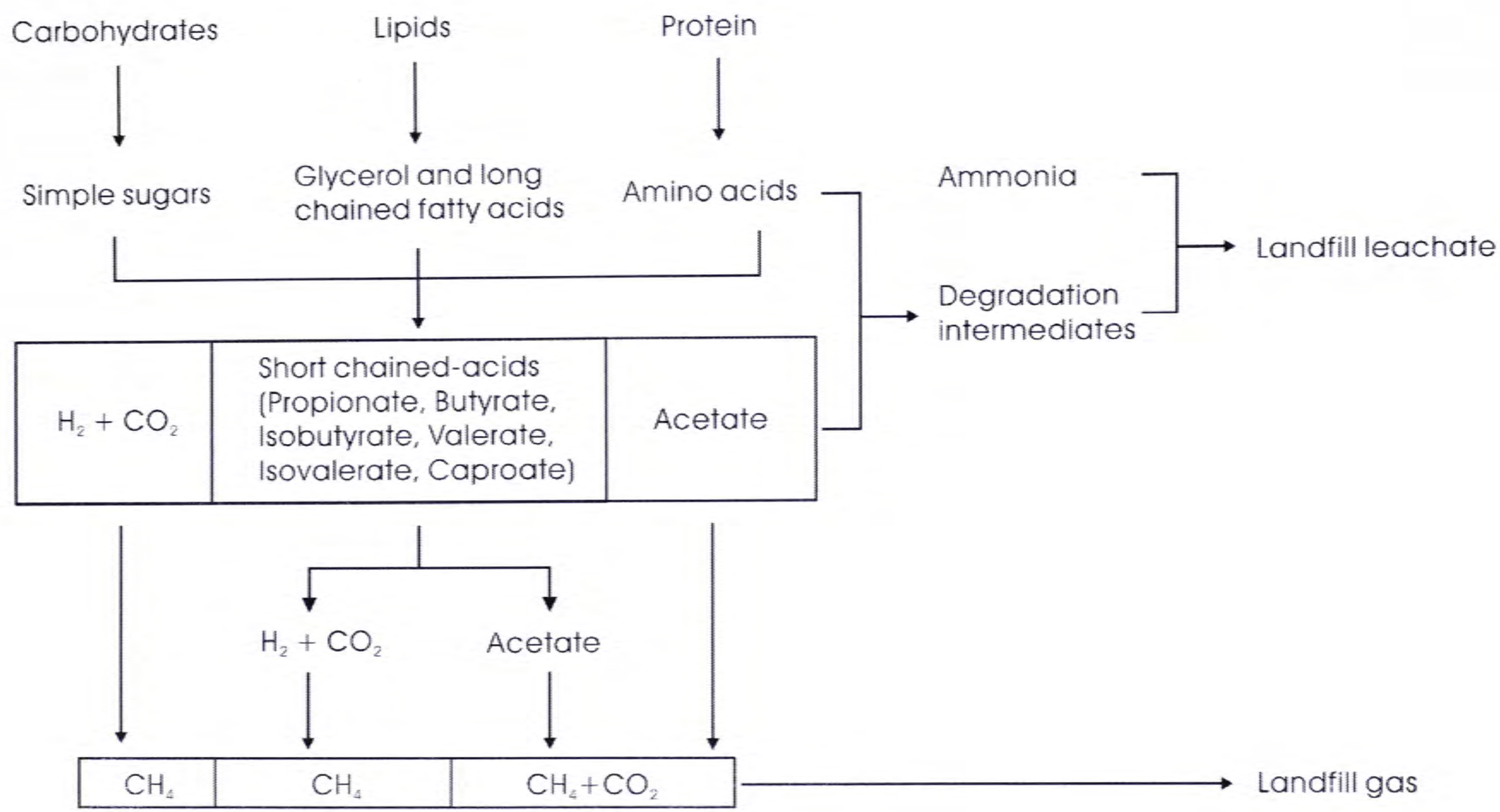


Figure 1.3 Processes of anaerobic decomposition in landfills (redrawn from Department of the Environment, UK, 1986).

The aerobic phase is very short because of the limited amount of oxygen in landfills and the relatively high oxygen demand of wastes. The local environment becomes anaerobic, encouraging the growth of facultative anaerobic microorganisms. Volatile organic acids and carbon dioxide are produced in the anaerobic acetogenic phase. When the population of methanogenic bacteria build up, volatile acids produced in the acetogenic phase, and other organic compounds, are converted to methane and carbon dioxide. Air (mainly N_2) is displaced from the void space. Eventually a dynamic equilibrium is reached with a gas ratio of approximately 60% methane to 40% carbon dioxide. The methanogenic phase can last for tens of years. The rate of degradation slows down as the substrates are depleted.

1.2.2 Control of degradation by-products

A sanitary landfill requires careful design and operation plus good aftercare to ensure the sanitary and economical disposal of solid wastes. The earlier natural attenuation landfills were designed on the concept that the leachate would be attenuated by the soil beneath the landfill and diluted by underground aquifers (Figure 1.4a). This type of landfill usually caused serious groundwater contamination even after many years since the closure of the landfill.

Modern landfills are based on the concept of containment. Landfills of this type may be single- or double-lined depending upon the site specific requirement so that leachate does not seep into the groundwater (Figure 1.4b). The containment principle also requires a high degree of design and engineering, such as leachate and gas collection systems, to control over hazards associated with the degradation by-products

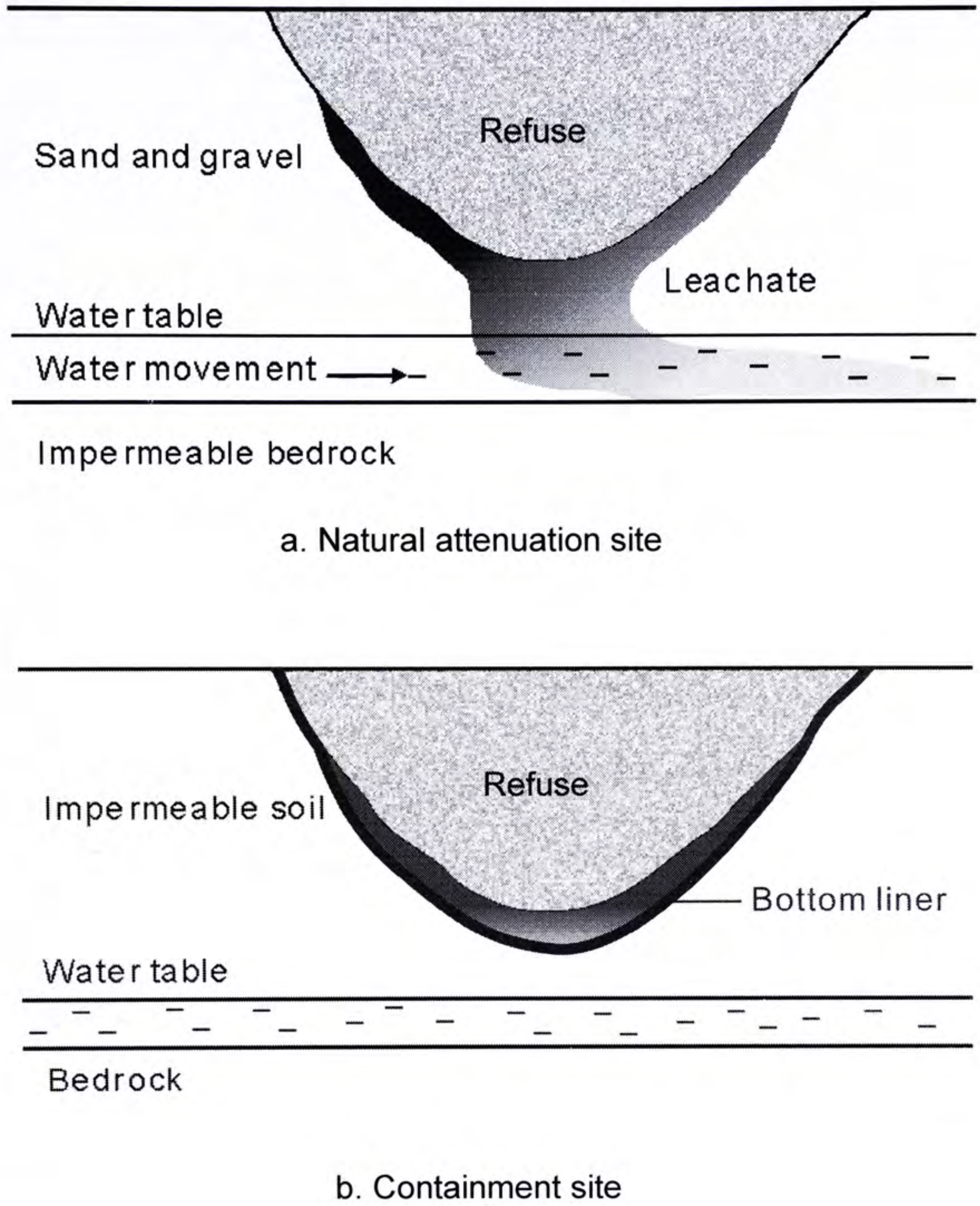


Figure 1.4 Schematic diagrams illustrating the dispersion of leachate under landfills of (a) natural attenuation and (b) containment design. The relative concentration of leachate is shown by the darkness of shaded areas (redrawn from Qasim and Chiang, 1994).

(Figure 1.5). A drainage system is installed to prevent rainfall and groundwater from entering the wastes. The generation and release of pollutants are kept to a minimum. The entombment landfill is based upon the principle of a containment landfill, but with attempts to further prevent the infiltration of liquids (rainfall and groundwater) (Westlake, 1995); wastes inside are stored in a relatively dry form.

Increased awareness of the long-term liabilities associated with landfills was led to a greater need for control of the landfill after completion of the operation phase. Landfills after closure must be maintained over many decades. Post-closure care involves maintaining the integrity and effectiveness of the final cover, operating the leachate collection and treatment system, the gas collection system and environmental monitoring. The final end use of the site varies according to the local environment conditions and community needs.

1.3 Landfill leachate

Prior to the 1960s, few people were aware of the fact that water passing through solid wastes in a sanitary landfill would become highly contaminated. This wastewater, termed leachate, was generally not a matter of concern because few cases of water pollution were reported where leachate had caused harm. It is now known that leachate from sanitary landfills may be an important source of water pollution (Boyle and Ham, 1974).

1.3.1 Generation and control of landfill leachate

Leachate is generated as a consequence of the contact of water with the waste

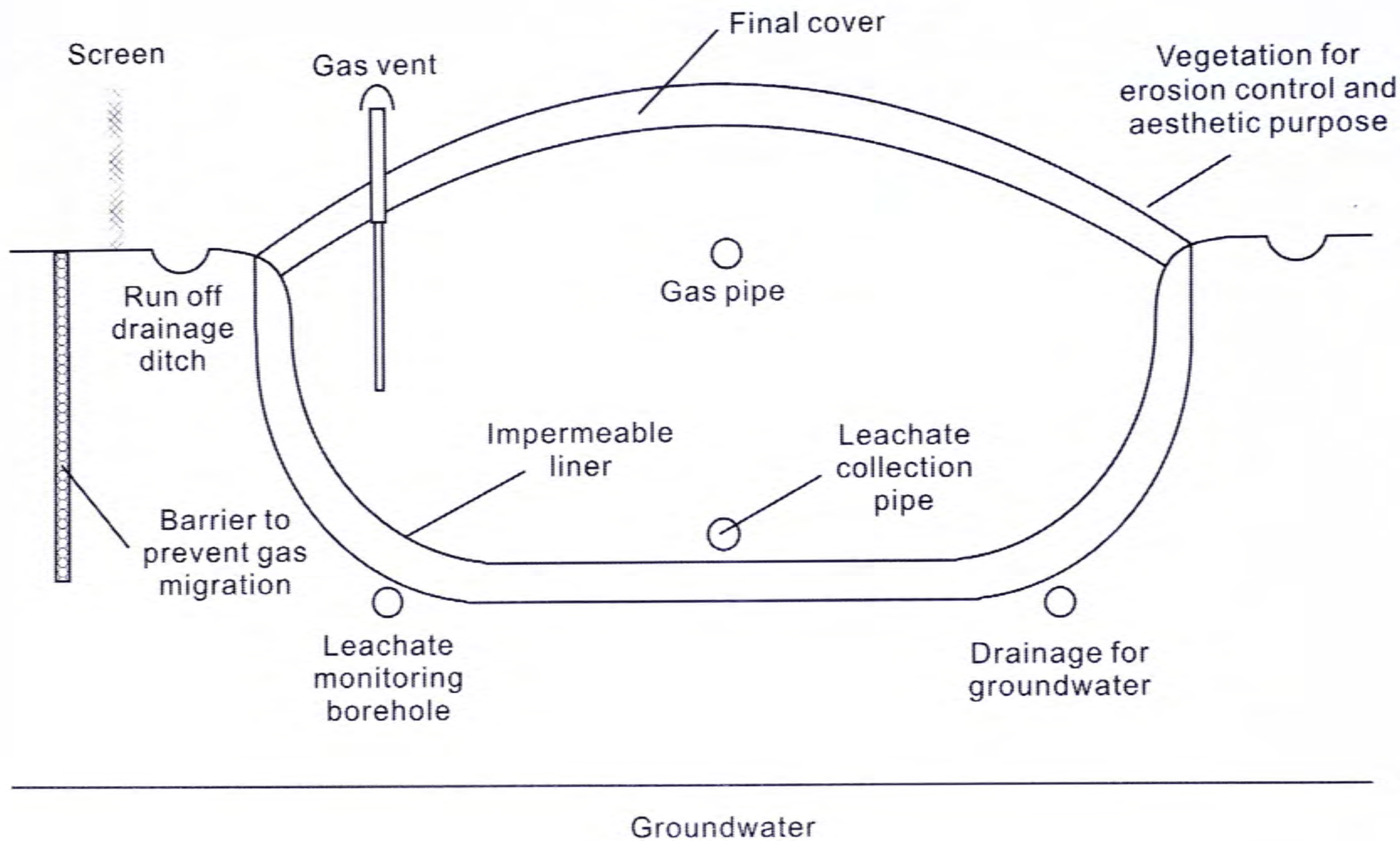


Figure 1.5 Cross section of a modern containment landfill. On-site leachate treatment facilities such as ammonia stripping plant and aeration tanks are not shown. In some landfills with high gas production rate, landfill gas is collected to generate power for on-site usage. Surplus gas is flared in the flaring plant (redrawn from Reinhart and Townsend, 1998).

mass. It may contain soluble or suspended materials in wastes, as well as byproducts of waste degradation. The sources of water are primarily precipitation, irrigation, and runoff which infiltrate through the landfill cover; groundwater intrusion and the initial moisture present in refuse. A landfill water budget analysis can be used to predict the amount of leachate produced. A simplified water budget for a landfill is presented in Figure 1.6.

The control of landfill leachate can be accomplished by either source control or end-of-pipe control. Generally as more water flows through the solid wastes, more pollutants are leached. The volume of leachate is minimized by limiting water infiltration through careful contouring and drainage design on the final cover, an impermeable liner beneath the landfill cap, and in some cases the proper selection and maintenance of a vegetative cover.

Leachate is confined within the landfill by a bottom liner system. It must be transported from the liner by a leachate collection system to minimize the pressure head on the liner. It is then pumped to an aboveground storage tank or buffer lagoon before treatment.

1.3.2 Leachate characterization

The composition of leachate among landfills is highly variable. Leachate contains higher pollutant loads than many raw industrial wastewaters. The variation in leachate quality can be attributed to differences in waste composition, landfill design and operation, and precipitation.

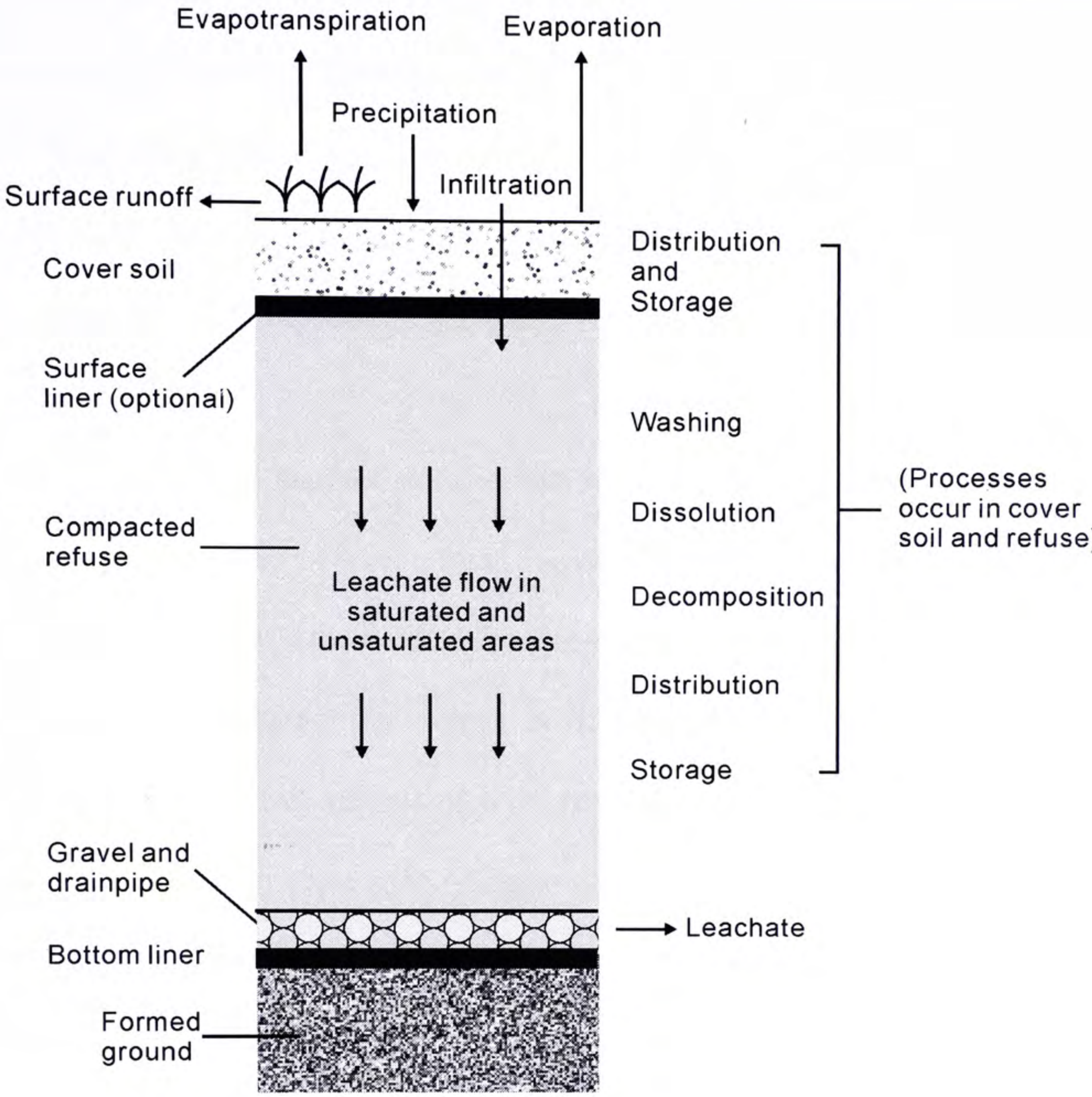


Figure 1.6 Moisture components at a sanitary landfill and the major processes involved in the formation of landfill leachate (redrawn from Qasim and Chiang, 1994).

Landfill leachate in general contains high concentrations of organic and inorganic contaminates (Table 1.1) (Pohland and Harper, 1986). Peak concentrations of COD and total solids exceeding 40000 mg L^{-1} are common. High concentration of contaminants is observed near the onset of leaching from young landfills, while dilution and microbial utilization of organics reduce leachate strength in older landfills.

The change in leachate composition with the various stages of fermentation follows a similar pattern (Table 1.1 and Figure 1.7) (Robinson, 1991; Andreottola and Cannas, 1992). Leachate produced in the aerobic phase dissolves highly soluble salts, such as Cl^- and others. As oxygen is depleted, organic acids produced in the acetogenic phase reduce the pH of leachate to 4-5. Metals become more soluble, along with elevated concentration of dissolved organic acids, and the ionic strength of leachate increases. The presence of organic acids also contributes to a high COD. The redox potential falls, reflecting an anaerobic condition. The redox potential continues to decrease in the methanogenic phase. The pH of leachate starts to rise when organic acids are assimilated by methanogenic bacteria. At the early stage of the methanogenic phase, the ionic strength is still high as materials are solubilized in decomposition. The strength of leachate gradually decreases with the age of landfill. Lu *et al.* (1984) developed a set of rate equations to describe the relationship between landfill age and some constituents in leachate within a limited time range. This information helps landfill practitioners design leachate treatment facilities for the changing volume and strength of leachate.

Table 1.1 Landfill leachate composition ranges as a function of the degree of landfill stabilization (Pohland and Harper, 1986).

Parameter	Stage of degradation		
	Acetogenic	Methanogenic	Maturation
pH	4.7 - 7.7	6.3 - 8.8	7.1 - 8.8
Electrical conductivity ($\mu S\ cm^{-1}$)	1600 - 17100	2900 - 7700	1400 - 4500
BOD ($mg\ O_2\ L^{-1}$)	1000 - 57000	600 - 3400	4 - 120
COD ($mg\ O_2\ L^{-1}$)	1500 - 71000	580 - 9760	31 - 900
Volatile acid (as acetic acid) ($mg\ L^{-1}$)	3000 - 18800	250 - 4000	0
NHx-N ($mg\ L^{-1}$)	2 - 1030	6 - 430	6 - 430

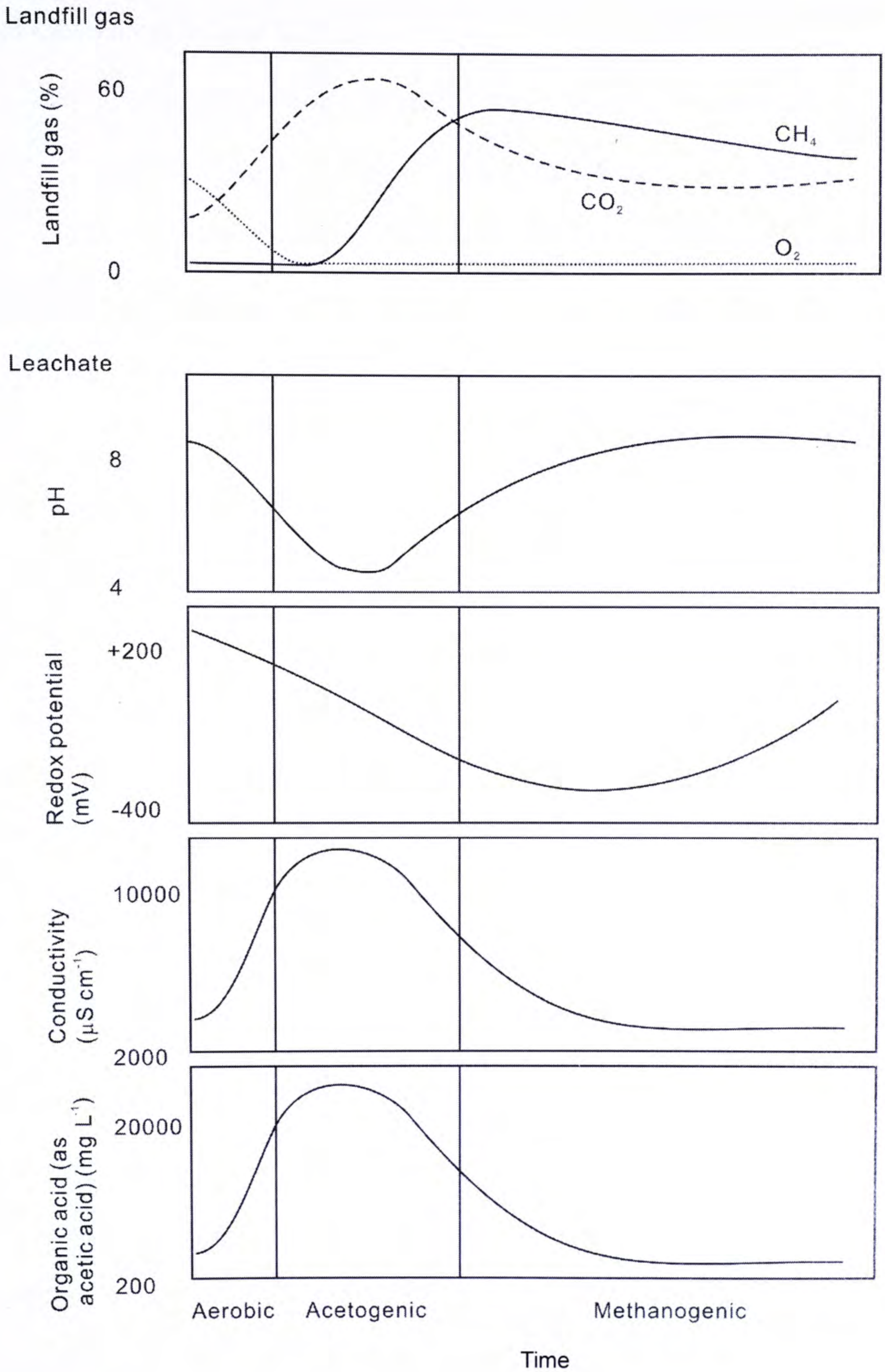


Figure 1.7 Changes in the composition of landfill gas and leachate during the course of waste degradation (adapted from Andreottola *et al.*, 1992; Pfeffer, 1992; Tchobanoglous *et al.,* 1993).

1.3.3 Leachate from local landfills

Patterns of decomposition in local landfills are similar to landfills in temperate regions (Figure 1.7), except that the methanogenic conditions are established much more rapidly. A high value of COD is found in the first year of filling. The aerobic and acetogenic phases are not clearly defined. These phases are expected to complete in the first year. Chen *et al.* (1997) reported that in the first year operation of the North East New Territories (NENT) Landfill, the COD of raw leachate decreased from about 70000 to 10000 mg L^{-1} indicating rapid change from the acetogenic phase to the methanogenic phase. Such an early establishment of the methanogenic phase in local landfills is probably attributed to the warmer climate, more rainfall and a larger portion of readily-degradable organic matter in the wastes such as domestic wastes and animal slurry in Hong Kong, which accelerate the rate of waste decomposition (Robinson, 1991; Lo, 1996).

1.3.4 Leachate treatment

Landfill leachate has long been recognized as a potential source of surface and ground water pollution. It is essential to collect and treat the leachate before discharge in order to safeguard the ecosystems. Physical, chemical and biological processes which are traditionally used for the treatment of municipal sewage can be used for leachate treatment.

However, designing leachate treatment facilities is more challenging than those for the treatment of municipal sewage. It requires knowledge about landfill design, temporal fluctuation of leachate quality and degree of treatment needed. Leachate treatment facilities are designed to provide service over the landfill life expectancy (over decades). Landfill leachate initially is a high-strength wastewater, characterized by high organic loading, NHx and sometimes by the presence of toxic chemicals. The volume and the composition of leachate will change with landfill maturation. Treatment processes must be sufficiently flexible to cope with these changes.

In Hong Kong, landfill leachate is treated with municipal sewage in municipal sewage treatment works. Co-treatment is a convenient method. Research suggests that some constituents such as NHx, COD and metals in leachate may impair the treatment process of biological treatment systems, resulting in high sludge volume, settling problems, foaming and poor effluent quality (Qasim and Chiang, 1994; Li and Zhao, 1999). Leachate can be co-treated with municipal wastewater at a level of 5% v/v. To prevent overloading in sewage treatment works, leachate from local landfills is pretreated on-site by ammonia stripping or aeration, or both, before co-treatment with municipal sewage.

1.4 Leachate irrigation

Although advanced treatment technologies are available, landfill operators seek alternatives because of their high capital cost and special management requirements. Land treatment of municipal wastewater is a proven technology which can be applicable to landfill leachate. It provides the possibility of nutrient reuse, and produces effluent of high quality. Land-based systems may also be used in conjunction with a conventional system in polishing final effluents.

1.4.1 Common practices of wastewater irrigation

A great variety of application methods are available. Irrigation may be by spraying, overland flow and rapid infiltration (surface application) (Figure 1.8). Methods such as wetland disposal, peat filter bed, subsurface irrigation and groundwater recharge are being tested. This section will focus on surface application methods.

1.4.1.1 Spray irrigation

Spray irrigation means controlled spraying wastewater on land. Surface runoff is held to a minimum. A large portion of water is lost by evaprotranspiration. Pollutants are retained in the soil profile and are attenuated. Spray irrigation can be applied in all types of topography. Soil erosion or waterlogging can be prevented by adjusting the application rate carefully.

1.4.1.2 Rapid infiltration

Rapid infiltration has been described as infiltration-percolation or groundwater recharge. Water is applied by sprinkling or, more common, by flooding techniques. Soil cover for this method should be well drained, with a depth of a few meters. Wastewater flows down rapidly and soil attenuates pollutants.

1.4.1.3 Overland flow

Wastewater is applied over the upper reaches of a slope and allowed to flow overland, which is collected at the toe of the slope. Overland flow is similar to spray irrigation but with infiltration being kept to a minimum. Water is forced to flow

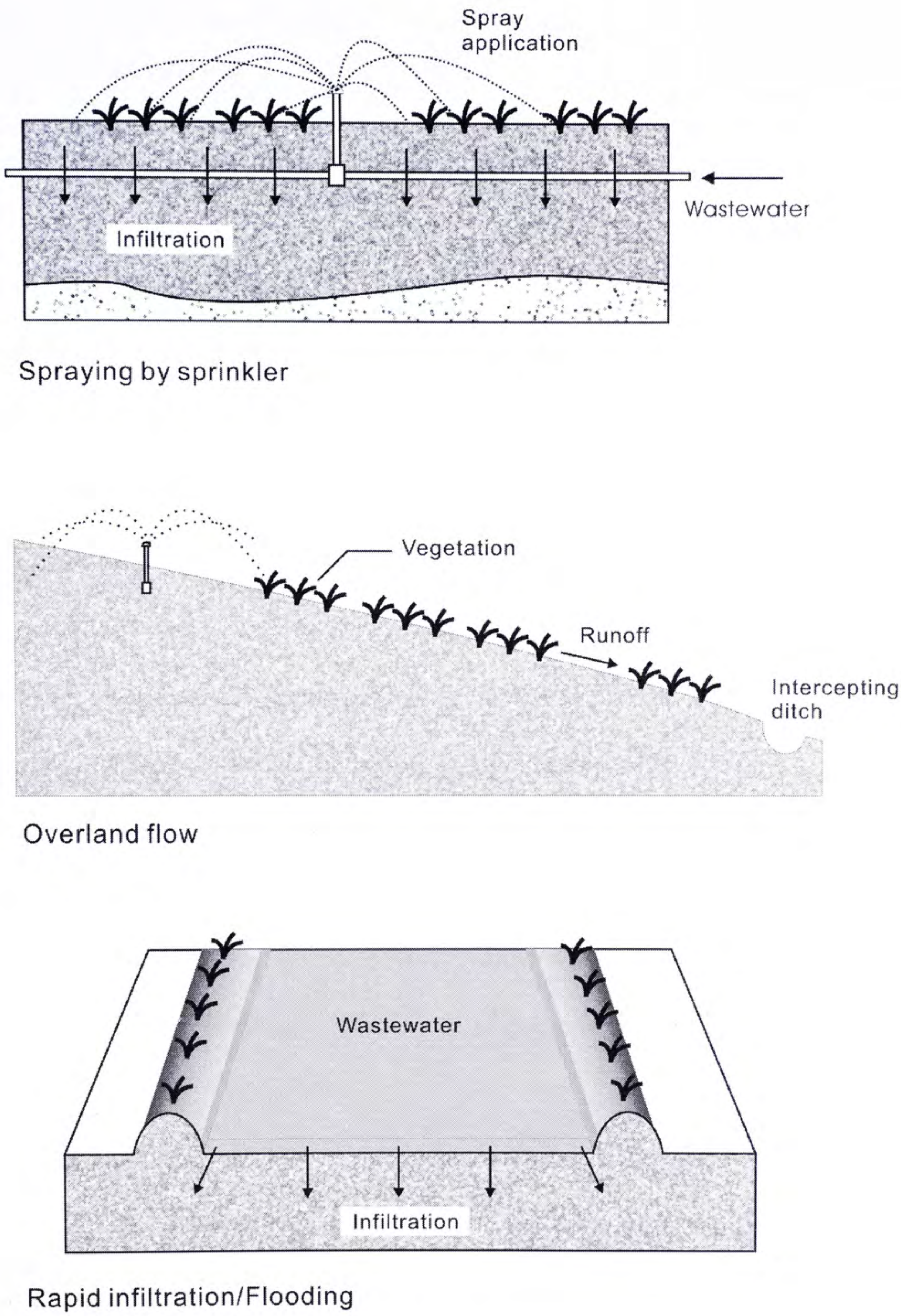


Figure 1.8 Wastewater application to the land for treatment purposes.

along the vegetated soil surface for pollutant remediation. Overland flow is the least effective among the three methods described. It requires sufficient retention time to accomplish attenuation. Slopes may be susceptible to soil erosion.

1.4.2 Effects of leachate irrigation

1.4.2.1 Effect of leachate irrigation on soil percolate

Soil is found to be effective in reducing metals, N and P in the leachate applied. The level of N decreased after passing through a soil-plant system (Xia *et al.*, 1999; Tyrrel, 2002). For metals, Fuller and Warrick (1986) demonstrated that the levels of heavy metals in leachate after passing through a soil column were lower than those in the raw leachate, indicating that attenuation by soil was taking place. The treatment efficiency may vary with soil properties and the depth of solute transport. Clay and loamy soils retained metals better than sandy soils due to higher ion exchange capacity and better retention of suspended solids (Fuller and Warrick, 1986; Wong *et al.* 1990; Jiang *et al.*, 2000). On the other hand, loamy soil with better aeration had high removal capacity of COD and N (Jiang *et al.*, 2000).

The mechanisms and factors affecting leachate decontamination are discussed by Bagchi (1987) and Qasim (1994). The attenuation of leachate by the soil-plant system is accomplished by three key components and processes (Hasselgren, 1992).

- *Soil*: Suspended solids are filtered and dissolved salts are retained by ion exchange, adsorption or precipitation.
- *Soil organisms*: Soil microbes stabilize organic substances and transform N in

leachate.

- *Plants*: Nutrients are taken up and incorporated into the biomass. Vegetative cover also helps reduce soil erosion and maintain soil structure which is favorable to the infiltration of wastewater.

Besides decontamination, land disposal, followed by evapotranspiration, can greatly reduce the amount of landfill leachate to be sent to treatment facilities. However, land treatment of landfill leachate is still limited to evaluation on an experimental basis. Soil salination, the tolerance of plants, sustainability of treatment efficiency and the risk of pollution through runoff and leaching become the major constraints of the extensive use of soil-plant systems for the treatment and disposal of landfill leachate.

1.4.2.2 Effect of leachate irrigation on soil

Landfill leachate contains considerable amounts of dissolved salts. Land application gradually increased the soil electrical conductivity significantly (Wong and Leung, 1989; Hernández *et al.* 1999).

Leachate irrigation in general increases the content of major cations such as Na and K (Winant *et al.*, 1981; Wong and Leung, 1989; Hernández *et al.* 1998). Fe and Mn, which are sometimes present at high levels in leachate, may be retained in the soil (Wong and Leung, 1989). Accumulation of metals may depend on the soil type, texture class, and organic matter content as well as the abundance of metal in leachate and the application method. Clay and loamy soils retain metals better. The affinity of cations to soil is: $Na^+ < NH_4^+ < K^+ < Ca^{2+} < Mg^{2+}$ (Chan *et al.*, 1978; Chan, 1982) and $Ni^{2+} < Cd^{2+} < Zn^{2+} < Pb^{2+}$ (LaBauve *et al.*, 1988). However, the accumulation of toxic heavy metals like Cd and Pb may not be observed, especially when they are present in trace concentration in leachate or the leachate is diluted prior to irrigation.

Nitrogen (N) is an essential nutrient for plants, and is usually added in large quantity to agricultural lands to obtain high crop yield. Application of municipal wastewater and landfill leachate resulted in the addition of considerable amounts of N to the soil (Winant *et al.*, 1981; Wong and Leung, 1989). Effluent N will eventually enter the N cycle. It is taken up by plants, transformed in soil and lost in gaseous form or via leaching. The details of these processes are discussed in Chapter 4.

Contrast to municipal sewage, the amount of P is low or even below the detection limits in some landfill leachate. Application of leachate may lead to a slight increase in soluble P in soil. However, it declines rapidly, due to adsorption and precipitation reactions. P is relatively immobile in soil; leaching and runoff loss is negligible.

There is extensive research on the toxicity of leachate to aquatic organisms. However, there is little information on the response of soil fauna and microbes to landfill leachate. Chan *et al.* (1999) reported that leachate application would inhibit the formation of root nodules and symbiotic N fixation in the roots of *Acacia confusa* and *Leucaena leucocephala.* On the other hand, a field study demonstrated that leachate irrigation increased the landfill gas emission and the methane oxidation activity in landfill cover soil (Murice *et al.,* 1998). However, the direct effect of leachate on soil microbial activity and the nutrient mineralization is still unclear. Wastewater provides a carbon source for soil microbes, enhancing the enzyme activity in soil (Madejón *et al.*, 2001).

1.4.2.3 Effect of leachate irrigation on plants

Leachate carries many intermediates and products of waste degradation. There is a concern, however, that the benefits of leachate irrigation may be offset by the presence of inhibitory chemicals. A number of research have identified some impacts such as leaf injury, yield reduction and poor survival rate.

Sub-irrigation (flooding) of saline MSW landfill leachate to 1-year old red maple saplings (*Acer rubrum*) resulted in turgor loss in the upper nodal leaves and reduction in the transpiration rate immediately after the first application. Over a 25-day treatment period, the photosynthetic rate decreased to about 50% of the control (deionized water treatment) (Shrive and McBride, 1995).

High salt concentration suppresses growth rate, resulting in stunted plants. Plants growing in saline soil developed a "waxy", dark blue-green appearance and thickening of the leaves (Leung, 1985). In more severe cases, symptoms like chlorosis and necrotic lesions or tip burn may be observed. In extreme conditions or when plants are sensitive to specific ions (e.g. Cl^-), leaf burn or even complete crop loss would occur. The mechanism for the yield reduction due to high salinity is still unclear. It may be the result of a general disturbance in metabolic processes, or a damaging effect on plant membranes which are responsible for cellular osmotic adjustment.

Plants may be variably sensitive to salts at different growth stages. In the past, because of the failure in seedling emergence in saline conditions, germination stage was thought to be sensitive. It has been shown that plants may not be more sensitive to salinity or phytotoxicity during germination. Many plants are tolerant during germination but are sensitive immediately after germination or emergence. Damage from salinity during early seedling development could be irreversible or fatal (Mass, 1993).

Short-term variation in leachate composition may severely affect the health of the vegetative cover and may add uncertainty to the leachate irrigation plan. The effect of salinity will also be more pronounced when evaporative demand exceeds the precipitation. The osmotic stress experienced by plants is greater as salts built up in the root zone. Moreover, saline wastewater may cause direct injury to plants. When water is applied by sprinkling, especially under dry weather condition, direct contact of the water drops with the canopy and the subsequent evaporation cause salt deposition on leaves and direct injury to tissues.

The most prevalent ion in leachate is Cl^-. It causes specific damage in some plants. Cl^- had a rapid effect on the growth and evapotranspiration of *Salix viminalis* (Stephens *et al.*, 2000). Plants irrigated with > 200 mM (7.1 mg L^{-1}) Cl^- showed turgor loss and bronzing of foliage after 1 day. The trees progressively lost their leaves during the experiment. The reduction in yield was significantly correlated with the tissue content of Cl^-. Usually leaf injury was caused by the direct wetting of the leaf by wastewater. Another cause of Cl^- toxicity is thought to be reduction in NOx uptake, resulting in nutritional imbalances (Feigin *et al.*, 1991).

In contrast, there was research which demonstrated the beneficial effects of leachate irrigation on plants. Application with diluted or low strength leachate may stimulate plant growth. The yield of *Brassica chinensis* and *Brassica parachinensis* was stimulated when being irrigated with diluted leachate (5 - 20% v/v) (Wong and Leung, 1989). There was a significant correlation between the foliar N content and leachate concentration. Moreover, irrigation with diluted, low strength leachate enhanced the growth, survival and stomatal conductance of *Acacia confusa, Leucaena leuocephala* and *Eucalyptus torelliana* (Liang *et al.*, 1999).

1.5 Landfilling in Hong Kong

1.5.1 Climate

Hong Kong has a sub-tropical climate. Under the influence of a monsoon climate, Hong Kong has a distinct hot humid summer and a cool dry winter. January and February are cloudy, with occasional cold fronts followed by dry northerly winds. It is not uncommon for the air temperature to drop below 10°C in urban areas. May to August is hot and humid; afternoon temperatures often exceed 31°C whereas at night, temperatures generally remain around 26°C with high humidity. The mean annual precipitation is about 2200 mm. About 80% of the rain falls between May and September (HKO, 2003a).

1.5.2 Geography and economy

Hong Kong is situated at the southeastern tip of China. With a terrestrial area of 1100 km^2 and a population of 6.8 million in 2003 (CSD, 2003), the average population density of Hong Kong is one of the highest in the world. Land is at a premium in Hong Kong. Owing to the rapid increase in population and development needs, the civil and construction industry has also been growing with the land and property price. As a result, a large quantity of construction wastes has been generated and has become a major waste management problem.

1.5.3 Waste composition

Figure 1.9 shows the composition of solid wastes in Hong Kong. Municipal solid wastes (MSW) include solid wastes from households, industrial and commercial sources. They comprised to about 45% of the wastes dumped in landfills. However, industrial wastes comprise only 1% of the total solid wastes (EPD, 2003a).

The growth in municipal wastes has been compounded many times by the huge amounts of construction wastes going to landfills. They include the wastes arising from such activities as construction, renovation, demolition, land excavation and road works. Although 76% of these wastes are recovered, mainly for use in reclamation, a significant portion of the wastes still goes to landfills. In 2002, construction wastes made up nearly 50% of all wastes going to landfills, pushing up the total wasteload (EPD, 2003a).

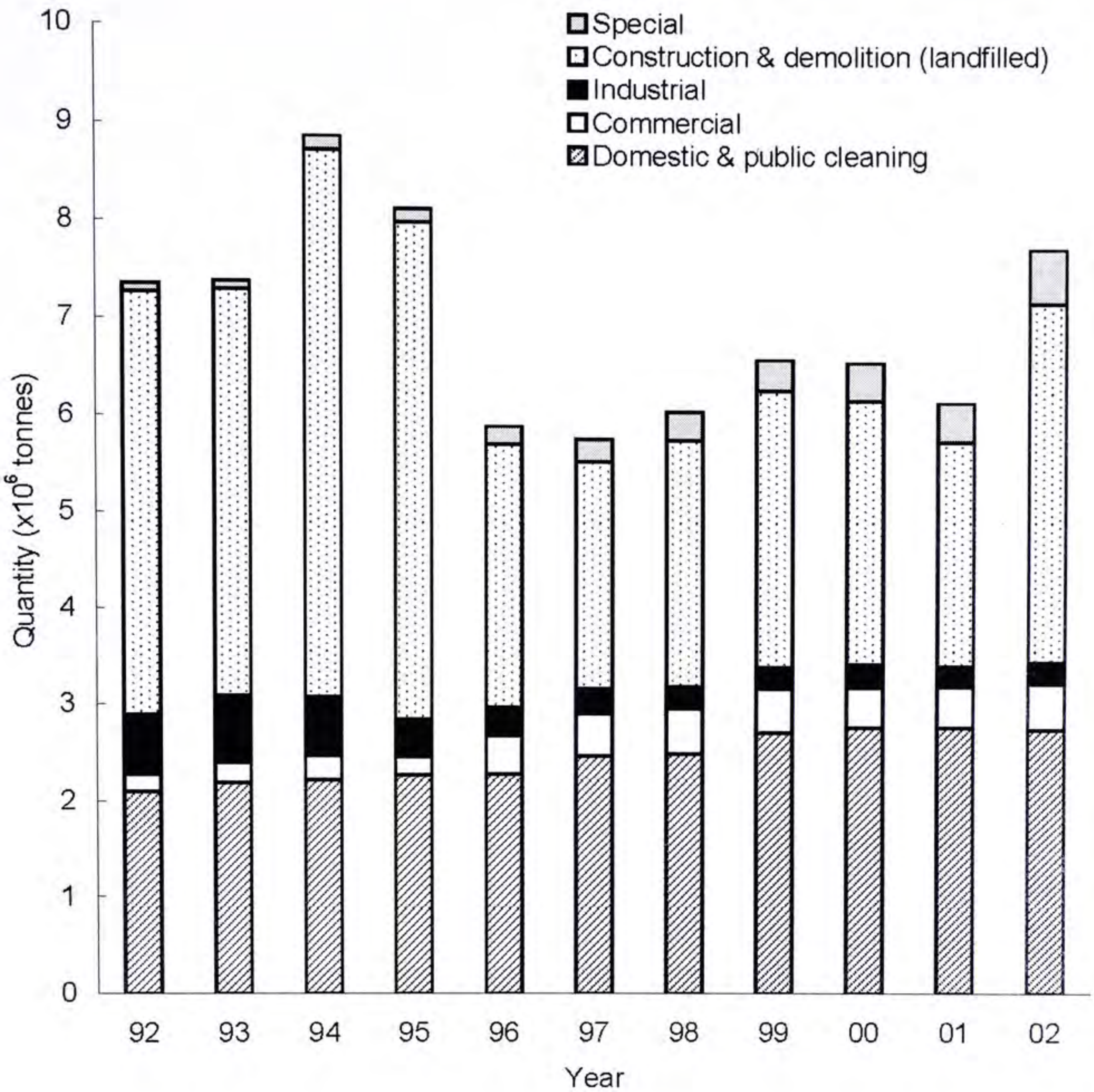


Figure 1.9 Composition of solid wastes in Hong Kong in 1991 - 2002 (EPD, 2003a).

Notes: Municipal solid wastes (MSW) comprise solid wastes from household, commercial and industrial sources, but exclude construction and demolition wastes, chemical wastes and other special wastes.

1.5.4 Leachate sampling sites

Since the 1950s, the Hong Kong Government has been providing landfills for the disposal of solid wastes. In the past, landfill sites were built simply to bury wastes. The closed landfills were built based on the dilution and attenuation design, with little control over landfill gas and leachate.

In the 1980s, the Government started planning large and modern landfills with high environmental standards to meet the growing waste disposal demand and to safeguard the health and the welfare of the community (EPD, 2002). Three strategic landfills with a total capacity of 135 million tonnes have been built and have become the sole means of waste disposal. They are of containment design equipped with a double lining system, as well as landfill gas and leachate collection systems (EPD, 2003b). Leachate is also pretreated on the site prior to co-treatment with municipal sewage.

After the commission of the three modern landfills, the 13 closed landfills, occupying a total land area of 300 ha or 1.6% of the urban area, were progressively restored to minimize potential safety and health risks. Final capping, landfill gas management systems and leachate treatment works have been installed (Figure 1.10). To recover the valuable land space, some restored landfills are landscaped to provide green zones and are developed into public recreational uses (Table 1.2) such as golf driving ranges (Plate 1.1).

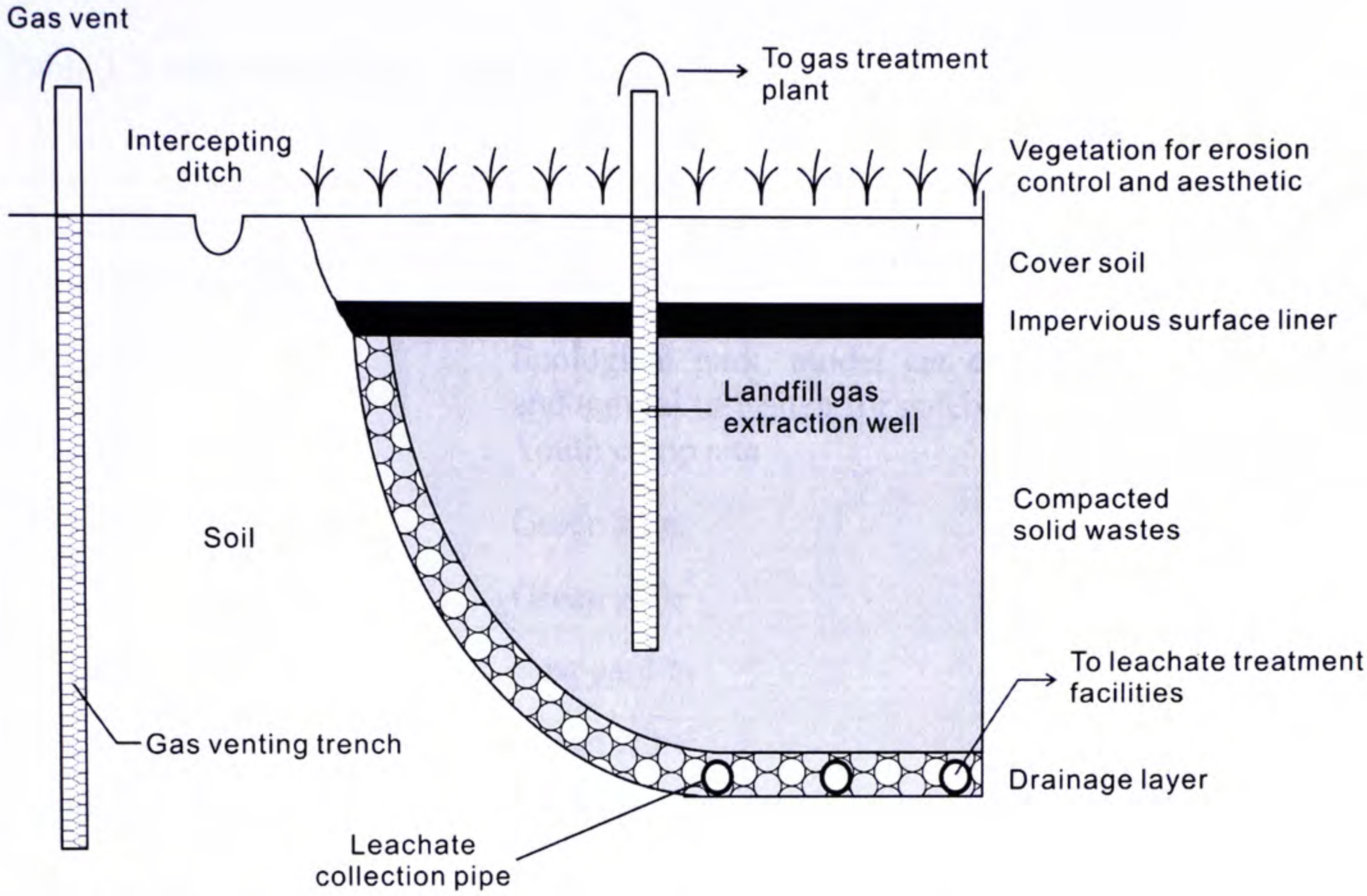


Figure 1.10 Typical cross section of a restored landfill (EPD, 2003b).

Plate 1.1 (Left) The aerial photo of Shuen Wan Landfill. Closed in 1995 and restored in 1997, Shuen Wan Landfill has been landscaped to form a golf driving range (right) for use by the general public since 1999 (EPD, 2003a).

Table 1.2 Afteruse of some local landfills (EPD, 2003a).

Landfill site	Proposed/ tentative afteruse
Gin Drinkers Bay	Green zone
Jordan Valley	Ecological park, model car circuit, education centre and natural turf pitch for gateball
Ma Tso Lung	Youth camp site
Ma Yau Tong Central	Green zone
Ma Yau Tong West	Green zone
Ngau Chi Wan	Rest garden
Ngau Tam Mei	Green zone
Pillar Point Valley	Currently under construction work
Siu Lang Shui	Green zone
Sai Tso Wan	Recreation ground for soccer and baseball
Shuen Wan	A 145-bay golf driving range has been open for use by the public since April 1999. Upgrading of the existing driving range to a 9-hole golf course being planned.
Tseung Kwan O Stage I	Multi-purpose grass pitches, model car racing tracks
Tseung Kwan O Stage II/III	Green zone

Leachates from five landfills of different ages were selected for the studies. The location and information of the landfills are shown in Figure 1.11 and Table 1.3. They can be classified by differences in age, waste composition and management practices. Raw leachate samples were collected from an unrestored landfill (Pillar Point Valley Landfill), two restored landfills (Ma Yau Tong Central and Tseung Kwan O Landfill) of different ages, and two operating landfills (West New Territories (WENT) Landfill and South East New Territories (SENT) Landfill). Although not well documented, the WENT Landfill receives mainly municipal solid wastes while a larger portion of construction wastes was dumped into the SENT Landfill. All leachate samples were taken at pipe outlets or sampling wells of leachate collection system, before entering the leachate treatment facilities.

1.6 Objectives of this study

1.6.1 Knowledge gaps

Landfill leachate has long been recognized as a source of water pollution. The environmental risk associated with landfill leachate is largely attributed to the high levels of NHx which threatens surrounding waters. Removal of NHx is an essential prcocess of leachate treatment. Research on leachate irrigation mainly focuses on the use of the soil-plant system for leachate treatment, by investigating and maximizing the treatment efficiency. The methods of leachate application, for example, the dilution level, were seldom determined in the light of the phytotoxicity data of individual leachate samples and this may lead to different responses. Moreover, there is little information on the beneficial uses of landfill leachate.

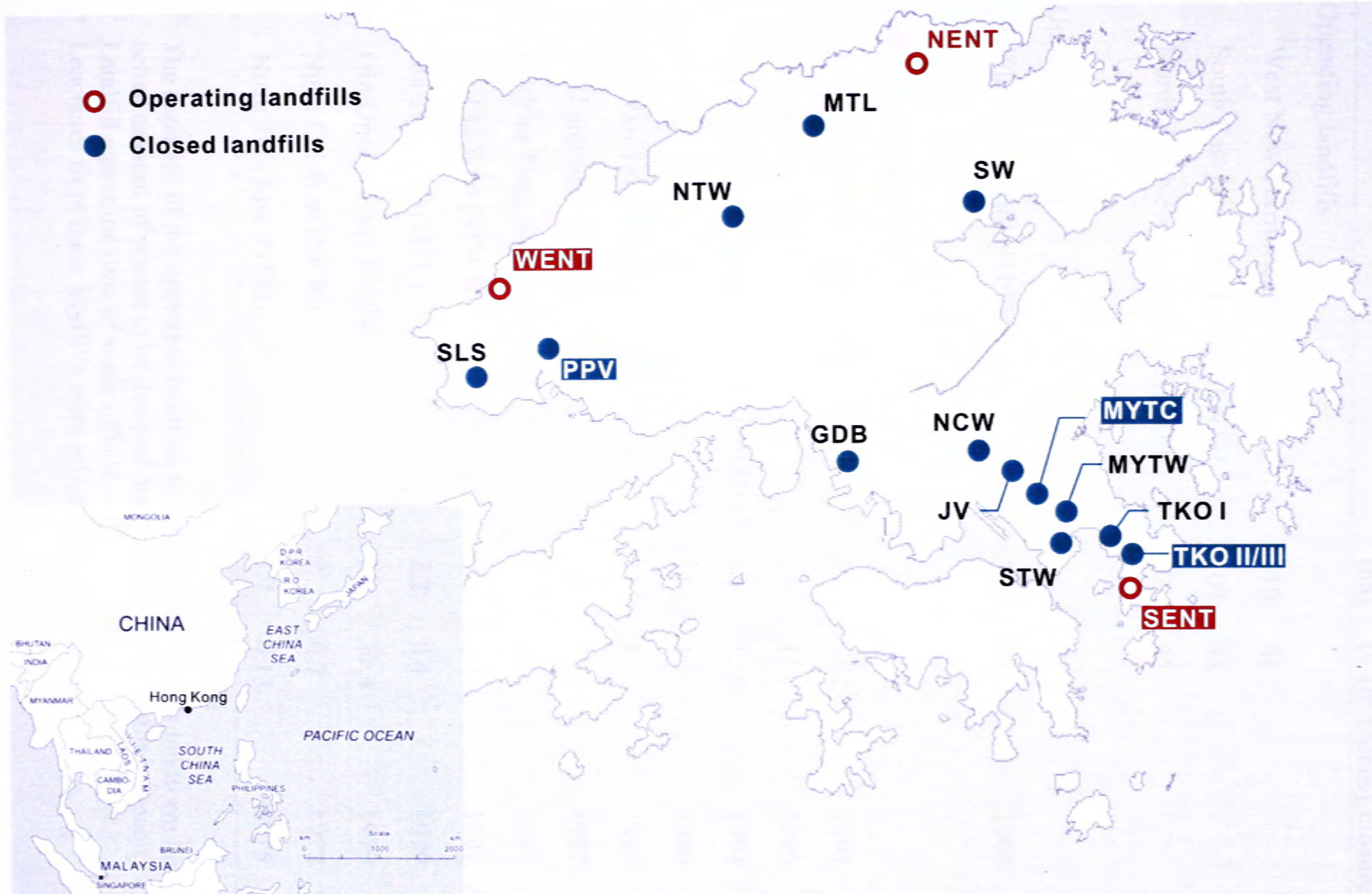


Figure 1.11 Location of sanitary landfills in Hong Kong and the location of Hong Kong in the Southeast Pacific Region (insert). Leachate from five landfills (with their name highlighted) were selected for the study (CSD, 2003; EPD, 2003b).

Table 1.3 Site information of the landfills in Hong Kong (EPD, 2003b).

Landfill site	Area (ha)	Capacity[1] (x 10^6 tonnes)	Year of closure	Age[2] (year)
Operating landfills				
West New Territories (WENT)*	110	61		
South East New Territories (SENT)*	100	43		
North East New Territories (NENT)	67	35		
Unrestored landfill				
Pillar Point Valley (PPV)*	38	13	1996	6
Restored landfills				
Tseung Kwan O Stage I (TKO I)	68	15.2	1995	7
Shuen Wan (SW)	50	15	1995	7
Tseung Kwan O Stage II/III (TKO II/III) *	42	12.6	1994	8
Jordan Valley (JV)	11	1.5	1990	12
Ma Yau Tong Central (MYTC) *	11	1	1986	16
Siu Lang Shui (SLS)	8.3	2.1	1983	19
Ma Yau Tong West (MYTW)	6	0.6	1981	21
Sai Tso Wan (STW)	9	1.6	1981	21
Ma Tso Lung (MTL)	2.2	0.4	1979	23
Gin Drinkers Bay (GDB)	28	11.4	1979	23
Ngau Chi Wan (NCW)	8	0.7	1977	25
Ngau Tam Mei (NTM)	1.7	0.13	1975	27

1 The capacity of the operating landfills is based on their latest landform design. The actual amount of wastes to be dumped may depend on the settling capacity of wastes.
2 Landfill age at the time of study (2002).
* Leachates from these landfills were selected for the study.

Landfill leachate does have problems, yet it represents a unique opportunity in wastewater utilization. Leachate contains considerable amounts of NHx and other nutrients and can serve as an alternative source of plant nutrients. Depending on the case, the cost of fertilizer and irrigation comprise of about 20 - 30% of wood production on landfills (Hasselgren, 1998). Utilizing landfill leachate in this way would provide a remarkable economical benefit in urban forestry programmes and the production of ornamental plants.

This study aimed at evaluating the feasibility of using landfill leachate as an alternative N source to fertilizer and employing phytotoxicology information to safeguard the plants in landfill leachate irrigation.

1.6.2 Project outline

In accomplishing the proposed objectives mentioned above, the study consisted of 3 experiments. Seed germination/root elongation tests were conducted to evaluate the phytotoxicity of leachate. This provided a general picture on the toxicity level of leachate samples (Chapter 2). A dilution factor, which is tolerable by plants, would be determined from the dose-response relationship. Twelve tree species were screened against leachate application at the EC50 level (Chapter 3). Species which showed good growth response with leachate irrigation would be selected for a subsequent experiment. Finally, a soil column experiment was carried out to compare the effect of leachate and artificial fertilizer on the plants as well as the distribution of nutrients in the soil-plant system (Chapter 4).

Chapter 2 Phytotoxicity evaluation of landfill leachate using seed germination tests

2.1 Introduction

Plants are primary producers, supporting almost all other living things on the earth. They play an active role in transferring contaminants to higher trophic levels. Because of their ecological importance, the potential of a chemical, or mixture of chemicals, to inhibit the growth of plants or to accumulate in plants should be considered when making environmental management decisions.

2.1.1 Tests involving the use of germinating seeds

The most widely used phytotoxicity test involving terrestrial plants is the seed germination test, which measures germination rate, root elongation, or other responses as endpoints. It has been used extensively for detecting the phytotoxicity of various samples such as sewage sludge (Pascual *et al.*, 1997), livestock waste compost (Tam and Tiquia, 1994), industrial effluents (Wang and Keturi, 1990), and landfill leachate (Devare and Bahadir, 1994). It is much simpler but more sensitive and efficient than using mature plants for phytotoxicity evaluation.

2.1.2 Importance of germination to plants

Germination is defined as the events associated with the initiation of embryo growth in a seed of higher plants. It includes many physiological processes such as activation of protein, translation of preformed RNA and cell expansion and division. Germination is the first step in plant development. Compared with older plants, development of young seedlings right after the germination is more susceptible to environmental stressors such as high salinity (Mass, 1993; Khan and Gulzar, 2003). Injury may be irreversible and may have impact on the survival of plants.

2.1.3 Advantages of germination tests

Phytotoxicity tests using germinating seeds have several advantages. Many dry plant seeds have a long shelf life. The cost of storage is negligible. It is an advantage over some tests in which the stock culture of test organisms is costly to maintain. Moreover, seeds can be activated at any moment, after adding water or after simple pretreatments, giving the test a permanent standby status. Some tests using plant seedlings can only be conducted in growing seasons when the seedlings are available seasonally.

The germination test is simple. It does not require sophisticated techniques and equipment. Results of germination tests can be obtained in a few days. They are very efficient compared with other growth studies using mature plants, which usually require weeks to complete. In addition, it requires only a small quantity of test sample; for example, a Petri dish design with 6 doses and 4 replicates requires less than 100 mL of sample. The amount of hazardous chemicals to be disposed of (or exposed to during handling) can be minimized. All these greatly reduce the cost of the phytotoxicity assay.

2.1.4 Limitations of using germination as an endpoint

The seed germination assay, often claimed as testing a sensitive and critical stage in the plant life cycle, may be insensitive in some circumstances. For example, the embryonic plant can derive its own nutrient requirement from internal reserve. Toxicants may not be taken into the seeds during germination. Moreover, the exposure time of germination test is short compared with many physiological and ecological processes (Table 2.1). It may be unable to detect the chronic effects on plants and ecosystems. Furthermore, from an ecological perspective, seed germination is relatively unimportant for perennial plants and species that do not require seeds to propagate. Conditions that can inhibit germination may not affect the survival of established plants.

2.1.5 Methods of germination tests

2.1.5.1 Test design

The term 'germination tests' generally describes bioassays that involve the use of germinating seeds. Germination test methods can be classified into two categories, root elongation tests and emergence tests. Root elongation tests are indirect tests; plant seeds are exposed to test substance hydroponically or on solution imbibed supports such as quartz sand and filter paper. In contrast, emergence tests measure toxicity associated with soil directly. The chemical is mixed with the reference soil or artificial support media to give a series of concentrations. Both types of test methods can be conducted at a single concentration to determine the intensity of effect or at multiple concentrations to develop a dose-response relationship. Of the various test types and designs to address germination, seed emergence tests using soil provide a more realistic type of exposure that can occur to seeds in the environment. Seed emergence tests can be conducted *in situ* to evaluate plant response in the field.

Table 2.1 Simplified conceptual model of the apparent timescales of physiological and ecological responses to chronic exposure to pollutants (adapted from Armentano and Bennett, 1992).

Response variable	Timescale interval
Pollutant uptake	10^{-1} - 10^{3} min
Reduced photosynthesis; altered membrane permeability	10^{1} - 10^{3} min
Germination, root growth and emergence	10^{0} - 10^{1} d
Reduced carbohydrate pool	10^{0} - 10^{1} d
Reduced growth of root tips and new leaves	10^{1} - 10^{2} d
Decreased leaf area	10^{2} - $10^{2.5}$ d
Differences in species growth performance	10^{2} - $10^{2.5}$ d
Reduced community canopy cover	10^{2} - 10^{3} d
Reduced reproductive capacity	10^{2} - 10^{3} d
Shifts in interspecific competitive advantage	10^{2} - $10^{3.5}$ d
Alteration of community composition	$10^{2.5}$ - 10^{4} d
Change in species diversity	10^{3} - 10^{4} d
Change in community structure	$10^{3.5}$ - $10^{4.5}$ d
Functional ecosystem changes (e.g. decline in nutrient cycling efficiency, net productivity)	$10^{3.5}$ - $10^{4.5}$ d

2.1.5.2 Plant species

There were 1569 plant species recorded in an early version of the PHYTOX database (Fletcher et al., 1988), of which only less than 30 species are recommended by the U.S. Environmental Protection Agency and the Organisation for Economic Co-operation and Development to be used routinely in seed germination tests (Table 2.2). Selection of these species can generally be attributed to several criteria (OECD, 2000):

- accessibility to characterized test species;
- ease of rearing in a testing laboratory;
- reproducibility of results within and across testing facility;
- economic and ecological importance (food, ornamental or major cash crops);
- sensitivity to many toxic compounds and having been used in previous bioassays;
- compatibility with the controlled environmental growth conditions and time constraints of the test method; and
- requiring no special pretreatment such as soaking, chilling, prewashing, light cycle and scarification.

2.1.5.3 Measurement endpoints

Suggested endpoints vary from quantitative measurement such as germination rate, survival, shoot length, root length and biomass to qualitative visual assessment. Percentage germination and root length are more popular endpoints in phytotoxicity assays. Germination index (GI) which combines seed germination and root growth can detect the inhibition of root growth under lower doses as well as the higher toxicity which may affect germination rate (Zocconi *et al.*, 1981). It has proved to be a very sensitive index in phytotoxicity determination.

Table 2.2 Species recommended for seed germination tests (USEPA, 1996; OECD, 2003).

Species	Common name	OECD	USEPA
Allium cepa	Onion	✓	✓
Avena sativa	Oats	✓	✓
Beta vulgaris	Sugar beet	✓	
Brassica alba	Mustard	✓	
Brassica campestris	Chinese cabbage	✓	
Brassica napus	Oilseed rape	✓	
Brassica oleracea	Cabbage	✓	✓
Brassica rapa	Turnip	✓	
Cucumis sativa	Cucumber	✓	✓
Daucus carota	Carrot	✓	✓
Glycine max	Soybean	✓	✓
Hordeum vulgare	Barley	✓	
Lactuca sativa	Lettuce	✓	✓
Lepidium sativum	Garden cress	✓	
Lolium perenne	Perennial ryegrass	✓	✓
Lycopersicon esculentum	Tomato	✓	✓
Oryza sativa	Rice	✓	
Phaseolus aureus	Mung bean	✓	
Pisum sativum	Pea	✓	
Raphanus sativus	Radish	✓	
Secale cereale	Rye	✓	
Secale viridis	Rye	✓	
Sorghum bicolor	Grain sorghum	✓	
Sorghum vulgare	Shattercane	✓	
Trifolium ornithopodioides	Fenugreek/Birdsfoot trefoil	✓	
Trifolium pratense	Red Clover	✓	
Triticum aestivum	Wheat	✓	
Vicia sativa	Vetch	✓	
Zea mays	Corn	✓	✓

It should be noted that there are many versions of the definition of germination in plant growth. Seed is considered to be 'germinated' when the length of primary root is equal or greater than 5 mm (USEPA, 1996). Kapustka (1997) suggests that germination completes when the seed coat is penetrated by the elongating embryo. On the other hand, 3 mm radicle was used as the operational definition of germination by the USFDA (1987). Results of phytotoxicity assays may not be comparable when the definitions of germination are different.

Conventionally, root and shoot lengths are measured manually. Wang and Williams (1990) suggested the use of root dry weight instead of root length as a measurement endpoint which can reduce the assessment time to approximately 10% of that required for measurement of individual root length. Innovation in image analysis technology provides a more rapid and reliable means of endpoint measurement. Image analysis technology was formerly developed for manufacturing industry and remote sensing. Algorithms compatible to germination tests have been developed recently (McCormac *et al.,* 1990; Kimura *et al.*, 1999). After incubation with test chemicals, an image of seedlings is acquired by a flatbed scanner. Root length is measured by counting the number of pixels on a processed image of a seedling. Root length determination in an image of 100 seedlings acquired at 600 dpi can be completed in seconds, with precision of less than 0.1 mm. Moreover, since the images can be saved for later processing, seed handling and root length determination can be dealt with separately. More samples or dosages can be handled in each batch of analysis.

2.1.5.4 Statistical analysis and test endpoints

In toxicity tests with a single dose, hypothesis testing is conducted to judge the significance of the responses. In cases where dilution series are used, toxicology studies often use mathematical models to transform the biological responses into a function of dose. These include (but are not limited to) probit analysis, linear regression and curve fitting to a logistic dose response curve. The functions express the toxicity test results as a probability of achieving an effect level. For example, the function can describe a concentration (or a range of concentrations) at which there is a 95% probability of observing a certain degree of effect such as growth retardation. Most of the conventional models require monotonic responses. Recently, models which are compatible with dichotomous results have been developed and these will be further discussed in later sections.

2.2 Objectives of study

In this study, the phytotoxicity of leachate from landfills of different ages would be evaluated by the seed germination and root elongation of two selected plant species. The use of phytotoxicity assay combined with analytical chemistry provides a complete assessment, not only of the amount of contaminants present, but also the influences of toxicants on the plant species concerned. Each method is valuable for answering different parts of the question regarding the leachate irrigation plan. Results of chemical analysis help to identify the potential cause(s), while phytotoxicity testing defines the magnitude of the problem. An attempt was made to suggest some potential constituents in leachate which may lead to growth inhibition and mortality of plants.

2.3 Materials and methods

2.3.1 Sample collection

Leachate samples were collected in November 2001 from the leachate extraction wells in the PPV and MYTC Landfills or the inflow pipe of leachate treatment plants at the SENT, TKO and WENT Landfills. The samples were stored in air-tight 1-L polyethylene and glass bottles and stored at 4°C.

2.3.2 Chemical analysis

Leachate samples were analyzed for pH and electrical conductivity (Jenway 4330 pH and Electrical Conductivity Meter, Essex, England). Total organic carbon was measured by the IR-combustion method using a TOC Analyzer (Shimadzu TOC5000A, Kyoto, Japan). Chemical oxygen demand (COD) was determined by close reflux colorimetry according to the Standard Method #5200D (APHA, 1995). Total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were analyzed by a SAN^{Plus} Segmented Flow Analyzer (Skalar, Breda, The Netherlands), after semi-micro-Kjeldahl digestion using Hg as a catalyst (Skalar, 1995). Total metals were measured by an inductively coupled plasma atomic emission spectrophotometer (ICP-AES) (Perkin Elmer 4300DV ICP-OES, Norwalk, USA) after digestion with concentrated nitric acid at 120°C (Method 3030E, APHA, 1995).

The samples were filtered through a 0.45 μm Millipore membrane filter before analysis for soluble salts. Ammoniacal nitrogen (NHx-N), oxidized nitrogen (NOx-N), ortho-phosphate phosphorus (PO_4^{3-}-P) and chloride (Cl^-) were analyzed by a SAN^{Plus} analyzer. Dissolved metals were determined by an ICP-AES.

2.3.3 Statistical analysis

The differences in chemical properties were analyzed by one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference test at P = 0.05 where appropriate.

2.3.4 Phytotoxicity assay

Raw leachate was diluted to a concentration series with MilliQ water. Petri dishes (9 cm diameter) were lined with a Whatman No. 1 filter paper which was moistened with 5 mL of diluted leachate. Seeds of *Brassica chinensis* (Chinese white cabbage) and *Lolium perenne* (perennial ryegrass) were obtained from local seed suppliers. Twenty seeds of each species were placed in each petri dish and there were four replicates for each treatment. The dishes were arranged in randomized blocks and incubated at 20°C in darkness. Germinated seeds were counted and primary root length was measured manually by a caliper after 4 days. Seeds were considered to have germinated when the radicle penetrated the seed coat (Kapustka, 1997). Germination index (GI) was determined according to Zucconi *et al.* (1981).

$$\text{Germination index (GI)} = \frac{G_i}{G_0} \times \frac{R_i}{R_0} \times 100 \qquad (1)$$

where, G_i = Germination rate in treatment (%)
G_o = Germination rate in control (water) (%)
R_i = Root length in treatment (mm)
R_o = Root length in control (water) (mm)

The median effective concentration (EC50) was calculated from the dose response relationship between GI and leachate concentration by the Brain-Cousens model (Brain and Cousens,1989).

$$f(x) = \delta + \frac{\alpha - \beta + \gamma x}{1 + \exp\left[\beta \ln(\frac{x}{\psi})\right]} \quad (2)$$

where ψ is the EC50 and γ provides an estimate of the rate of increase at low dose. Positive γ indicates the presence of hormesis and is reduced to a simple logistic model when $\gamma = 0$ (Schabenberger *et al.*, 1999).

2.4 Results and discussion

2.4.1 Leachate characterization

Table 2.3 summarizes the chemical composition of the leachate samples. They were characterized by high COD and NHx-N content. The pH of leachate was about neutral or slightly alkaline. Landfills in Hong Kong develop methanogenic condition much earlier than those in other temperate areas. Alkaline leachates with high NHx content were found in the two operating landfills (WENT and SENT Landfills). The accelerated decomposition may be attributed to the relatively hot and humid weather and the high portion of readily degradable materials in the refuse (Carville and Robinson, 1991; Robinson, 1991; Lo, 1996).

NHx is a plant macronutrient as well as a water pollutant. The concentrations of NHx in leachate ranged between 343 - 4790 mg L^{-1}. The great majority of TKN (77 - 99%) was in NHx, probably derived from the deamination of nitrogenous compounds. The N contents of raw leachate far exceeded the effluent standards of local authority (Department of Justice, 1997).

Table 2.3 Chemical properties of leachate samples (Mean ± SD).

	MYTC	PPV	SENT	TKO	WENT
pH	7.12	7.93	8.23	7.93	8.14
Electrical conductivity	2.70	4.50	15.2	7.80	19.9
COD	158 ± 1.40	466 ± 3.01	2570 ± 243	850 ± 0.00	7350 ± 130
TOC	9.82 ± 2.20	39.1 ± 1.33	761 ± 7.21	66.8 ± 1.52	1980 ± 11.4
COD:TOC ratio	16.1	11.9	3.38	12.7	3.71
Total Kjedahl nitrogen (TKN)	378 ± 20.5	614 ± 13.7	2520 ± 53.9	1110 ± 52.8	4830 ± 38.1
Ammoniacal nitrogen (NHx-N)	343 ± 12.4	474 ± 24.0	2440 ± 44.1	1050 ± 21.5	4790 ± 188
Oxidized nitrogen (NOx-N)	< 0.1	< 0.1	1.85 ± 0.00	< 0.1	1.28 ± 0.21
Total phosphorus (TP)	1.50 ± 0.34	3.12 ± 0.18	9.37 ± 0.00	1.72 ± 0.16	25.4 ± 1.45
*ortho*phosphate phosphorus (PO_4^{3-}-P)	0.78 ± 0.03	2.76 ± 0.06	8.47 ± 0.00	0.73 ± 0.18	24.3 ± 0.16
Chloride (Cl^-)	220 ± 0.25	559 ± 2.14	3790 ± 18.8	719 ± 5.69	3100 ± 16.1
Total metals					
Na	181 ± 2.88	576 ± 7.69	2200 ± 26.4	936 ± 10.7	2480 ± 19.3
K	127 ± 1.20	251 ± 1.20	1120 ± 10.6	458 ± 7.58	1910 ± 9.70
Ca	81.6 ± 4.51	34.7 ± 4.51	26.5 ± 4.08	41.9 ± 2.06	37.1 ± 4.58
Mg	20.5 ± 2.05	20.9 ± 2.53	43.4 ± 5.03	22.3 ± 2.53	78.7 ± 2.53
Cd	< 0.001	< 0.001	< 0.001	< 0.001	0.02 ± 0.00
Cr	< 0.001	0.07 ± 0.01	2.58 ± 0.01	< 0.001	5.41 ± 0.04
Cu	< 0.001	< 0.001	0.28 ± 0.04	< 0.001	< 0.001
Fe	21.5 ± 0.13	5.19 ± 0.02	3.75 ± 0.52	3.83 ± 0.01	3.70 ± 0.00
Mn	1.11 ± 0.06	8.46 ± 0.03	8.44 ± 0.03	4.97 ± 0.05	0.74 ± 0.00
Pb	<0.001	<0.001	0.55 ± 0.00	<0.001	1.48 ± 0.07
Zn	0.81 ± 0.08	1.31 ± 0.06	7.62 ± 0.01	0.68 ± 0.03	1.90 ± 0.09

All units in mg L^{-1} except electrical conductivity (mS cm^{-1}) and pH, SAR and COD:TOC ratio which have no units.

Table 2.3 (Cont'd) Chemical properties of leachate samples (Mean ± SD).

	MYTC	PPV	SENT	TKO	WENT
Soluble metals					
Na	191 ±3.00	587 ±8.86	1520 ±6.82	931 ±14.2	1830 ±30.1
K	78.8 ±1.11	227 ±1.80	829 ±8.33	450 ±5.57	1430 ±17.4
Ca	18.0 ±2.59	18.4 ±2.04	8.54 ±0.00	34.3±6.09	22.2 ±4.56
Mg	19.0 ±2.53	20.4 ±2.56	28.5 ±2.05	21.5±4.03	62.3 ±7.03
Cd	< 0.001	< 0.001	< 0.001	< 0.001	<0 .001
Cr	< 0.001	< 0.001	< 0.001	< 0.001	4.68 ±0.07
Cu	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Fe	3.67 ±0.00	9.19 ±0.00	1.79 ±0.00	3.04±0.53	2.96 ±0.08
Mn	0.41 ±0.08	1.43 ±0.06	0.14 ±0.05	3.98±0.04	0.57 ±0.03
Pb	< 0.001	< 0.001	< 0.001	< 0.001	1.25 ±0.04
Zn	< 0.001	< 0.001	1.81 ±0.01	0.61±0.09	1.19 ±0.03
Sodium absorption ratio (SAR)	31.4	94.2	250	125	199

All units in mg L^{-1} except electrical conductivity (mS cm^{-1}) and pH, SAR and COD:TOC ratio which have no units.

The levels of NOx-N were very low or even below the detection limits. The reducing conditions in methanogenic landfills do not favor nitrification. The redox potential of the leachate from the local operating landfills ranged from -193 mV to +63.6 mV (Wong, 2003). Nitrification is absent at a redox potential of -200 mV (Kemp *et al.*, 1990). Moreover, NO_3^- has a very high solubility and it is virtually not retained in soil. Once formed, it is readily leached out from the landfill. The level of NO_3^- was further reduced by denitrification, which converted NO_3^- to gaseous N_2O and N_2.

The leachates were low in P, having the highest total P content of only 24 mg L^{-1} in the WENT leachate. It was lower than leachate from landfills of comparable age (Qasim and Chiang, 1994). The major form of P that existed in the leachate was orthophosphate (PO_4^{3-}).

P is essentially immobile in soil and the landfill body. Under alkaline conditions, PO_4^{3-} ions quickly react with Ca to form insoluble tricalcium phosphate ($Ca_3(PO_4)_2$). In time, it further reacts to form hydroxyl-apatite ($[3Ca_3(PO_4)_2]\bullet Ca(OH)_2$), oxy-apatite ($[3Ca_3(PO_4)_2]\bullet CaO$), carbonate apatite ($[3Ca_3(PO_4)_2]\bullet CaCO_3$) and fluorapatite ($[3Ca_3(PO_4)_2]\bullet CaF_2$) compounds, which are thousands of times more insoluble than $Ca_3(PO_4)_2$ (Budavari *et al.*, 1996). Less than 0.01% of P in soil exists as soluble forms (Brady, 1990). A dilemma exists here. In the perspective of leachate treatment, soil can effectively remove P by land application of leachate. However, the extreme imbalance of P content and organic loading becomes a limiting factor in the biological treatment of leachate and the application of leachate for nutrient reuse. Addition of P to leachate has been a general practice to maintain an optimal BOD to P ratio to about 100:1 for effective treatment (Robinson and Maris, 1983). Similarly, irrigation with leachate may not be able to satisfy the P demand of plants. Soil amendments such as sewage sludge and artificial fertilizers may be required.

The high salt contents in leachates were reflected by the high electrical conductivity (3 to 20 mS cm^{-1}). Undiluted leachate is highly undesirable for use as irrigation water (Landon, 1991). Besides osmotic stress to plants, land application of undiluted leachate may add considerable amount of Na to the soil. Under high levels of exchangeable of Na in soil, clay particles are susceptible to swelling and dispersion, causing deterioration in soil structure and reduction in hydraulic conductivity (Rowell, 1996). The effect depends on the relative concentration of Na, Ca and Mg, which is rated by the sodium absorption ratio (SAR). A profound sodic effect would be observed when the SAR is greater than 18 (Landon, 1991).

$$\text{Sodium absorption ratio (SAR)} = \frac{[\mathrm{Na}^{+}]}{\sqrt{[\mathrm{Mg}^{2+}]+[\mathrm{Ca}^{2+}]}} \qquad (3)$$

where [X] is the concentration of cations.

The SAR of the leachates ranged from 31.4 (MYTC leachate) to 250 (SENT leachate); the levels of Mg and Ca were too low to ameliorate the sodic effect of leachate.

The concentrations of heavy metals were relatively low (< 1 mg L^{-1}), with the exception of Fe, Mn and Zn. The low levels of metals can be attributed to the reducing and alkaline condition of landfills. They were retained in the landfill as they were precipitated out with carbonate, hydroxide and sulfide.

2.4.1.1 Comparison among landfill sites

Although all leachate samples were characterized by relatively high levels of COD and NHx-N, their strength varied remarkably with landfill age. The COD of the MYTC leachate (158 mg L^{-1}) was only 2.1% of that in the WENT leachate (7350 mg L^{-1}). The strength of leachate in general decreased with the landfill age, with the exception of the SENT leachate. The NHx-N and COD of the SENT leachate were only half of these of WENT leachate. The SENT and WENT Landfills commenced operation in September 1994 and November 1993, respectively (EPD, 2003a). They have comparable area and capacity. Both landfills were open for waste dumping at the time of this study. However, the SENT Landfill received a greater amount of inert construction and demolition wastes which produced leachate of lower strength.

The ratio of COD to TOC at a given time may provide information on the type of organic constituents present. While TOC analysis directly assesses the C atoms present in organic compounds, COD provides a measure of the oxygen-demanding substances. COD:TOC ratio reflects the average amount of oxygen needed for oxidizing each carbon atom. A high COD:TOC ratio may indicate organic compounds that are easily oxidized (e.g. alcohols). Lo (1996) reported that the COD:TOC ratio of leachate decreased with the age of landfill, indicating that large organic molecules are broken down to smaller, more oxidizable intermediates during waste degradation.

However, a different trend was observed in this study. The COD:TOC ratio tended to increase with the landfill age, ranging from 3.38 to 16.1. This can be explained by the leaching of refractory organics which were left in the landfill when waste degradation in a landfill comes to maturation.

During waste degradation, the COD, TOC and COD:TOC ratio of leachates decrease with landfill age as the organic fraction in wastes are broken down into simple and more oxidized forms, yielding CO_2 and CH_4 as the final products. However, when the degradation process approaches maturation, the COD:TOC ratio may rise again since only the recalcitrant materials are left in the landfill. One example is lignin, the primary component of paper. Although lignin is not toxic, it is not metabolized by anaerobic bacteria, even under the most ideal conditions (Barlaz, 1989). These compounds, which have not been degraded in earlier stages, may have a relatively high COD:TOC ratio. They may be not the major constituent in leachate during acetogenic and early methanogenic stages of waste degradation. However, they become more dominant as most of the easily degradable organics are leached out or mineralized. Frimmel and Weis (1991) reported the change in the molecular weight distribution of leachate. The low molecular weight fraction declined with landfill age and high molecular weight fraction became dominant. The leaching of refractory organics with high molecular weight finally led to the increase in the COD:TOC ratio of leachate.

Compared with the landfills in temperate regions, landfills in Hong Kong require a much shorter time to stabilize owing to the warmer and more humid climate. The phenomenon of increased COD:TOC in leachate near landfill maturation can be observed much earlier. It should be emphasized that the COD:TOC ratio only reflects the characteristics of organic constituents in leachate. Leachate samples having a high COD:TOC ratio do not necessarily have a high organic loading.

The COD:TOC ratio should lie in the range of 0 to +4, which is within the range of the theoretical oxidation state of a carbon atom (-4 to +4). However, the leachates from three closed landfills had COD:TOC ratios much larger than the theoretical maximum. Interference from NO_2^-, S^- and Fe (II) may contribute to the deviation (Vogel *et al.*, 2000). Moreover, organically-bound N may consume dichromate in the COD analysis and lead to the increase in COD:TOC ratio. The larger the portion of organic N, the higher is the COD:TOC ratio.

2.4.2 Phytotoxicity assay

2.4.2.1 Dose response relationships

Germination rate in water is an important criterion of seed quality. Using seeds with high germination rate can increase the confidence in determining the intensity of the response to leachate toxicity. In this study, the minimum germination rate of *Brassica chinensis* and *Lolium perenne* were 88% and 90%, respectively.

The germination rate and root length are plotted against the concentration of leachate (Figure 2.1). Germination rate and root length in general decreased with increasing leachate concentration, with the exception of the low concentration ranges in some leachates. The dose response curves of root length had a greater slope than that of germination rate, indicating that root length was more sensitive to leachate toxicity.

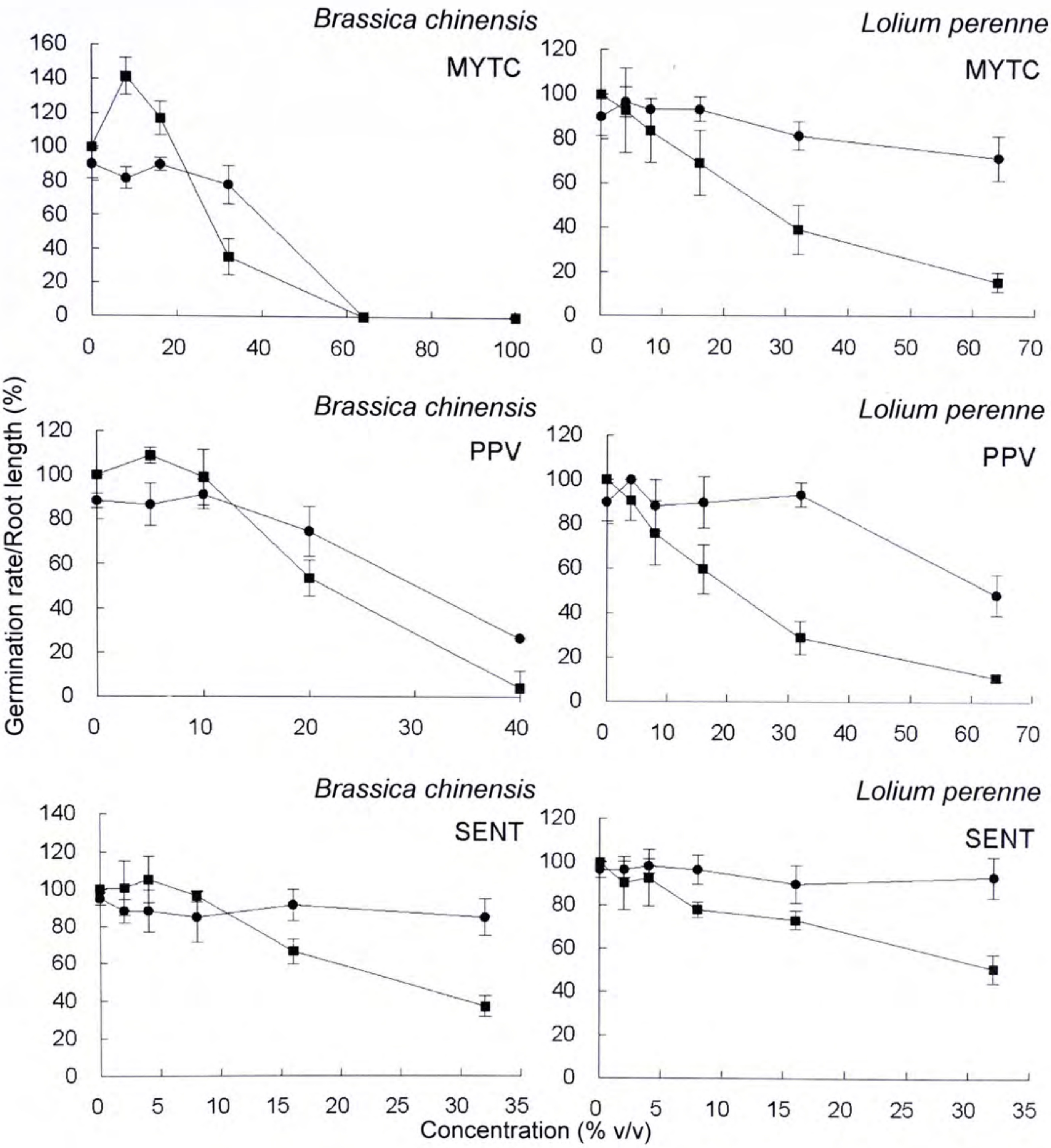


Figure 2.1 The germination rate (●) and root length (■)(as percentage of the control) of *Brassica chinensis* and *Lolium perenne* in the leachate germination test.

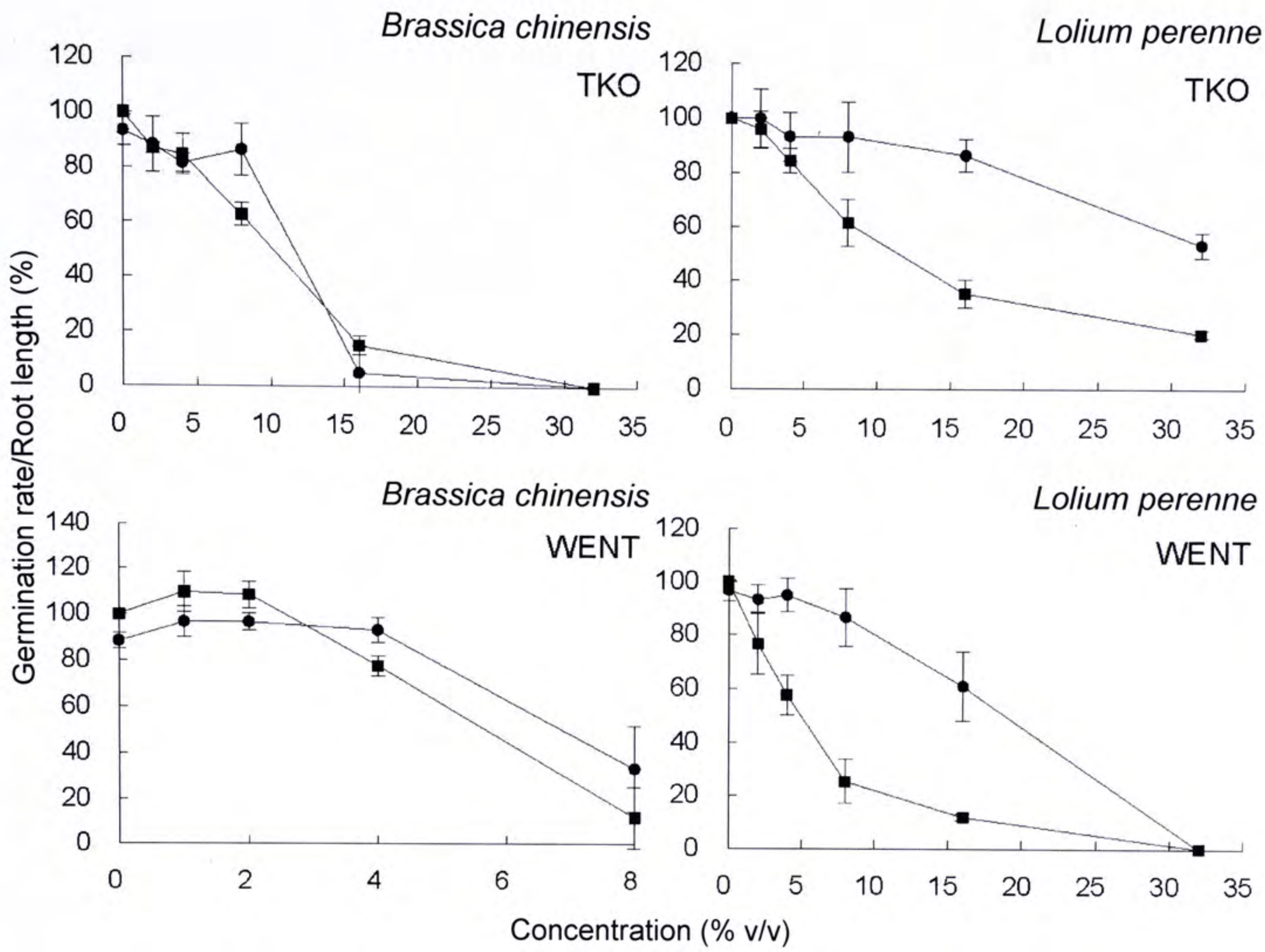


Figure 2.1 (Cont'd) The germination rate (●) and root length (■)(as percentage of the control) of *Brassica chinensis* and *Lolium perenne* in the leachate germination test.

Germination rate and root length were combined to yield a germination index (GI) (Zucconi *et al.*, 1981). Figure 2.2 shows the relationship between germination index and leachate concentration. Growth stimulation at low doses was observed in the dose response curve of some leachate samples. The germination indices in low leachate concentrations were higher that in the control (water only) treatment. The increase in the GI might be due to a large percentage germination and/or longer root. Similar findings were demonstrated in phytotoxicity tests of leachate (Leung, 1985) and livestock waste compost (Tam and Tiquia, 1994).

It is commonly accepted that the growth promotion is attributed to the presence of nutrient ions such as NH_4^+, K^+ in samples, provided that the toxic substances were diluted to a low level. In addition, hormesis may result in growth stimulation in low doses even in the absence of nutritional constituents.

Hormesis had an unusual history, in that the phenomenon was discovered since the19th century, and rediscovered more than once (Calabrese and Baldwin, 2000). It has not really gained general acceptance. Findings of hormesis were unwanted and unexpected since they lacked explicit mechanisms to account for it. However, it has been observed in numerous species from a broad range of taxonomic groups, chemical classes and biological endpoints. In a literature review by Calabrese and Baldwin (1997), 350 hormetic responses (8.7%) were identified in 4000 articles evaluated.

In most toxicity tests, the toxic effects are observed as the inhibition of some biological endpoints above a threshold level with the increasing concentrations of an

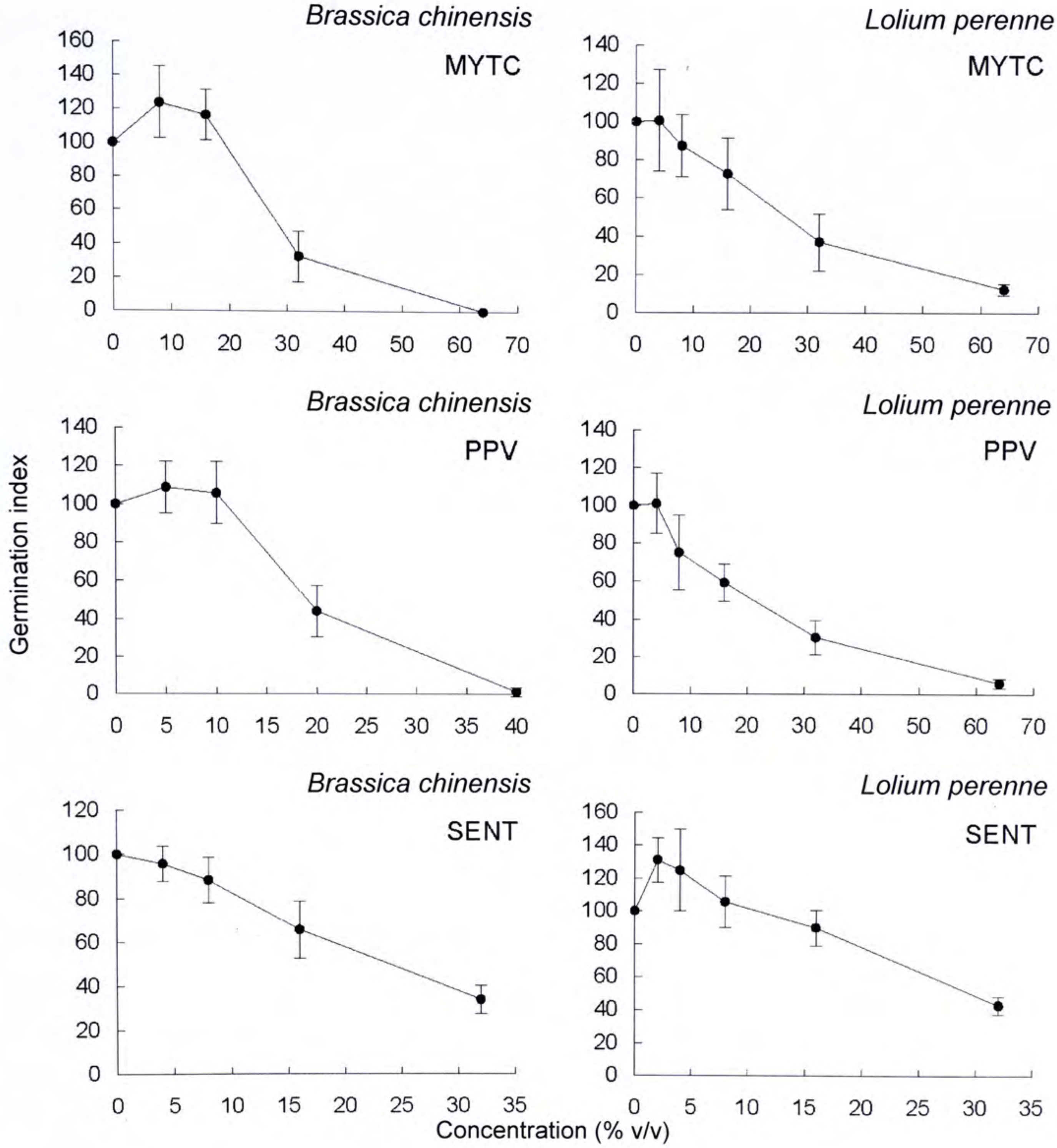


Figure 2.2 The dose response relationship between leachate concentration and the germination index of *Brassica chinensis* and *Lolium perenne*. Error bars shows the standard deviation of 4 replicates.

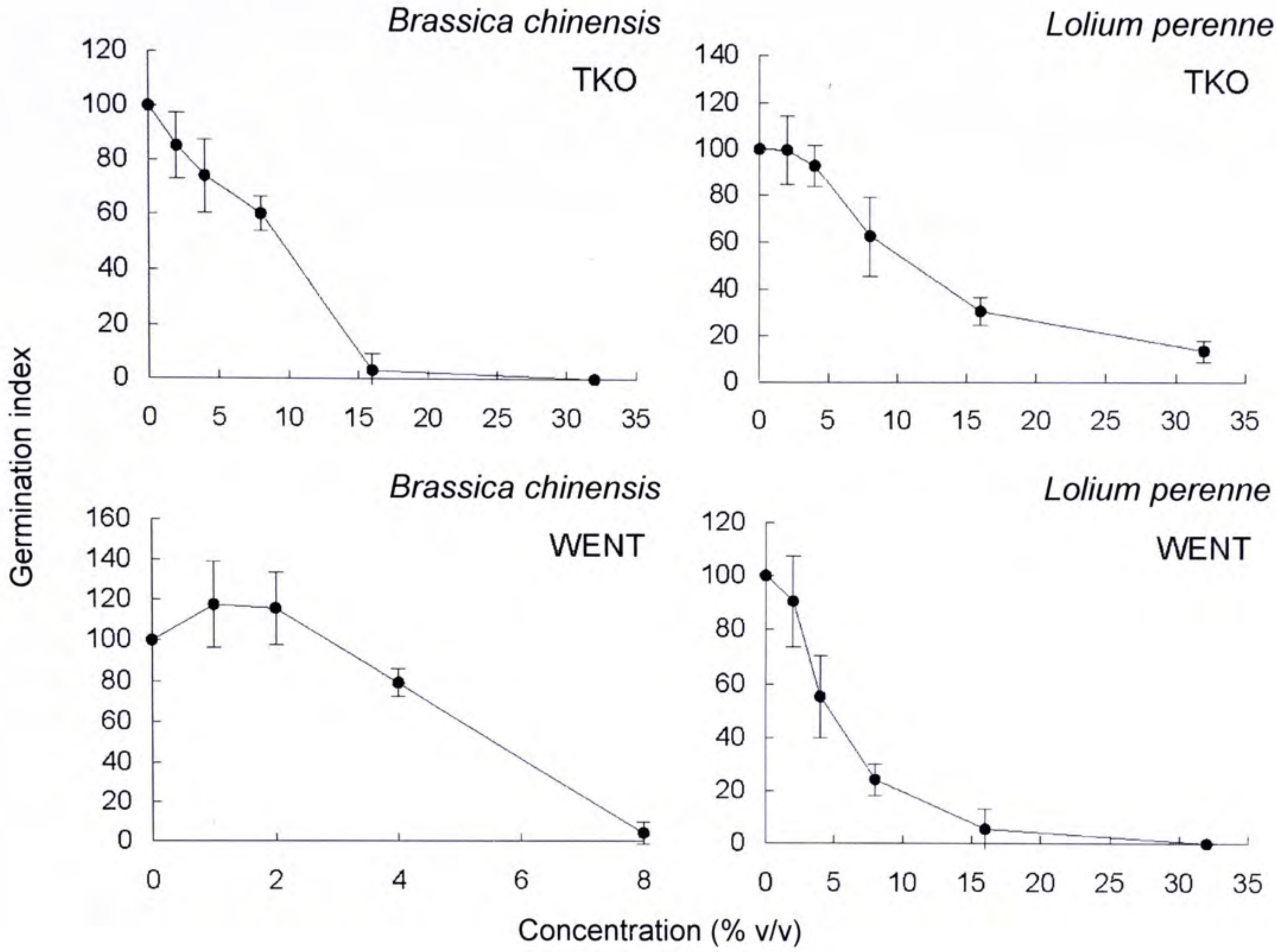


Figure 2.2 (Cont'd) The dose response relationship between leachate concentration and the germination index of *Brassica chinensis* and *Lolium perenne*. Error bars shows the standard deviation of 4 replicates.

inhibitory agent. There is another component in the relationship, called Arndt-Schulz Law or hormesis, which suggests that biological systems can be stimulated by low levels of inhibitors. An inverted U-shape pattern (or known as a beta-curve) can be observed in the dose response curves (Calabrese and Baldwin, 1998).

Stebbing (1998) suggested that hormesis is a normal outcome of biological systems that counteracts the effect of inhibitors and maintains a steady metabolic rate which is self-regulated by end-product inhibition (negative feedback mechanism) (Figure 2.3). Exposure to inhibitors would trigger the homeorhetic response to counteract the inhibitory load. Specific growth rate (SRG) increases to a maximum at first, and then oscillates until an equilibrium is restored (Figure 2.4). The transient over-correction in SRG brings about a cumulative increase in biomass, resulting in a hormetic response.

When the concentration of toxicants further increases, the onset of sustainable growth inhibition is observed. The beneficial effect (or the control mechanism in Stebbing's theory) is saturated by the overwhelming inhibitory load. When the response is plotted against the dosage, a beta-curve is obtained.

Although growth stimulation was observed, a conclusion of hormetic dose-response relationship cannot be made with confidence solely based on the findings of the present study. Calabrese and Baldwin (1998) suggested that hormetic response can be observed in 10-fold range below the no observable effect level (NOEL) and the intensity of response can be 30 - 60% higher than that of the control treatment.

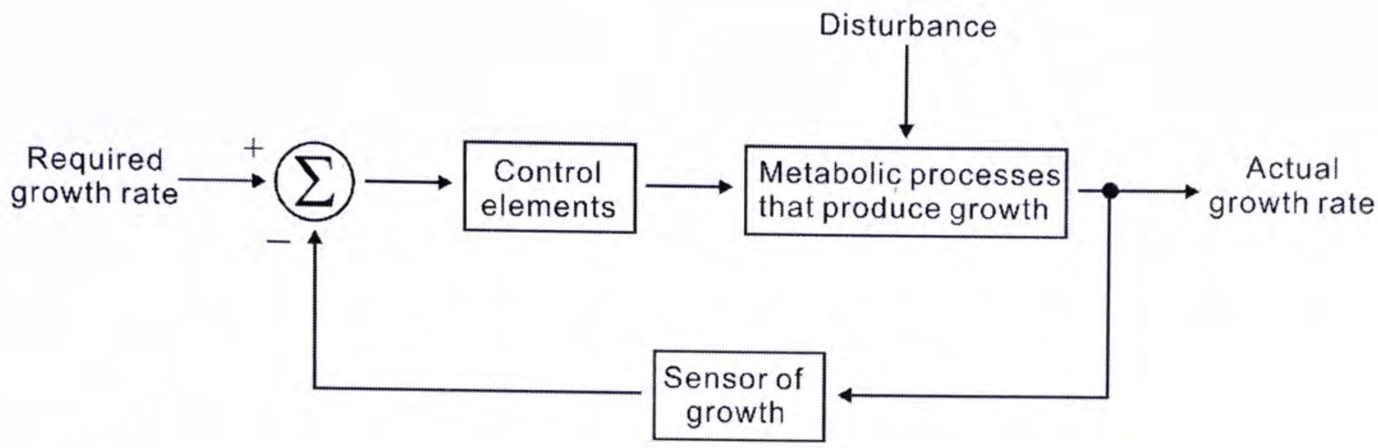


Figure 2.3 Schematic diagram showing the key features of feedback mechanisms for growth regulation (adapted from Stebbing, 1998). Disturbance (e.g. environmental stress) may affect the metabolism, which may result in the reduction in the relative growth rate. Change in metabolic rate is detected by a sensor element. Control elements adjust the metabolic rate in response to the signal from the sensor element and restore the actual growth rate to the required growth rate.

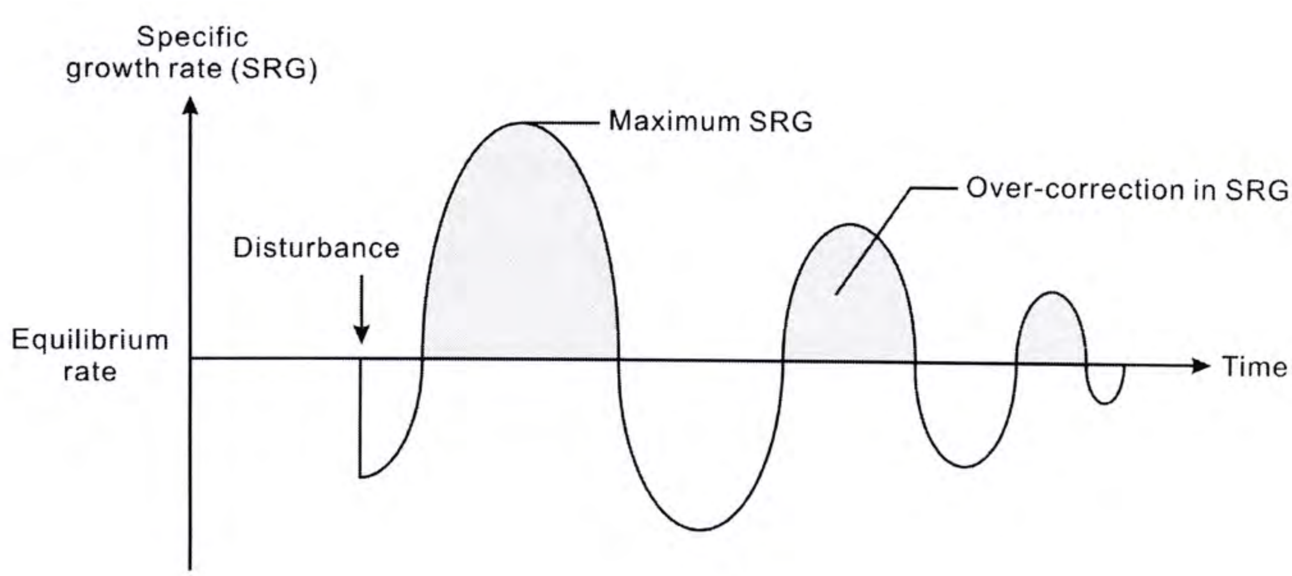


Figure 2.4 Conceptual diagram illustrating the change in specific growth rate (SRG) before and after exposure to inhibitor at a low dose. Before exposure to inhibitor, biosynthesis proceeds at the equilibrium rate. According to Stebbing (1998), inhibitor disturbs biosynthesis, leading to a decrease in specific growth rate. When homeorhetic process is triggered, the SRG increases to the maximum at first, and then oscillates until the equilibrium is restored. The transit over-correction in the SRG (shaded area) finally leads to the increase in cumulative growth.

This implies that four or more doses should be distributed in a specific manner below the NOEL in order to adequately assess the response in the hypothetical hormetic zone. The number of doses tested in this study was inadequate to describe possible hormetic responses. This study originally aimed at safety evaluation, which emphasizes the upper end (inhibitory zone) of the dose-response relationship. Only a limited number of doses can be tested in each toxicity test owing to time consuming endpoint measurement process.

However, the beta-curve pattern in the dose response curves should not be overlooked. As mentioned, the stimulation in root growth may indicate some beneficial effects of leachate on plants. A detail investigation in the dose response relationship may give some insights into the beneficial use of landfill leachate. The technical constraints of the root elongation test can be overcome by innovations in image technology which can greatly improve the efficiency of the endpoint measurement. More doses can be tested to assess the degree of responses near NOEL.

2.4.2.2 Implication of hormetic-like response on the selection of statistical model

Toxicology studies often use mathematical or statistical models to transform the biological endpoint into a function of dose. Probit analysis is the most popular statistical model to interpret bioassay results. However, some dose response relationships determined in this study have violated the assumption of the Probit model, which requires that the intensity of response should monotonically increase (or decrease) with the dose (USEPA, 1996). That is, the probit model cannot be used when there is a GI higher than that of the control treatment. A common practice is then to ignore a part of the data or to smooth the data. However, smoothing may lead to a biased estimation of the dose response relationship as the 'pooled' control response is larger than the true response in the control treatment. Brain and Cousens (1989) proposed a better solution by extending the logistic model so that it can accommodate both monotonic and dichotomous data. This model has been tested in toxicity test of herbicides (e.g. Schabenberger *et al.*, 1999) and it was adopted in this study for the calculation of the EC50s.

$$y = \frac{k(1+fx)}{1+e^{bg}x^{b}} \qquad (4)$$

The original form of Brain and Cousens model (Function 4) is unable to give the EC50 directly. Van Ewijk and Hoekstra (1993) parameterized the function (Function 2, in Page 44) to allow inference about quantities of interest.

2.4.2.3 Phytotoxicity of leachate samples

The EC50 of the leachate samples are presented in Table 2.4. Their values ranged between 4% (WENT leachate) and 30% (MYTC leachate) and in general decreased with the strength of leachate samples (Table 2.4). The WENT leachate (highest strength) was also the most phytotoxic among the 5 leachate samples. The EC50 of WENT leachate determined by *Brassica chinensis* was significantly lower ($P < 0.05$) than the other leachate samples.

There was a remarkable difference between the phytotoxicity of the leachate samples from the two operating landfill. The toxicity of the SENT leachate was significantly lower ($P < 0.05$), possibly due to the difference in their waste composition.

Table 2.4 The media effective concentrations of leachate samples tested with *Brassica chinensis* and *Lolium perenne.* The CI95 represents the 95% confidence interval of 4 replicates.

Leachate sample	EC50 (% v/v) ± CI95	
	Lolium perenne	*Brassica chinensis*
MYTC	23.2 ± 6.54 a	29.6 ± 2.41 a
PPV	18.5 ± 5.65 a	19.0 ± 1.40 b
SENT	17.5 ± 2.09 a	19.8 ± 6.10 b
TKO	9.19 ± 1.79 b	8.91 ± 0.61 c
WENT	4.29 ± 0.79 c	4.32 ± 2.43 d

When compared within a species, values in a column followed by the same letter do not differ significantly at $P > 0.05$.

The phytotoxicity of leachate could be primarily attributed to its high salinity. Electrical conductivity of 4 mS cm^{-1} would inhibit seed growth by disturbing water uptake in salt sensitive plants (Bewley and Black, 1994). Although *Lolium perenne* is more tolerant to salinity, yield reduction would be observed when the EC is higher than 5.6 mS cm^{-1} (Mass, 1993).

The osmotic effect of salts in leachate mainly impedes water uptake by plant roots by reducing the water potential of the substrate. The symptoms are turgor loss and wilting. Other effects of salt stress are delay in germination and emergence. It can be fatal if the seedlings, which are weakened by high salinity, encounter other stresses, such as an extreme temperature, synergistically (Mass, 1993). On the other hand, the effects of specific ions varied, as ions can interfere with physiological processes at different levels within an organism. Plants that are drought-resistant may be salt sensitive.

Cationic NH_4^+ can be utilized by plants as a N source, but becomes toxic when it is in excess. The effects of NHx on plants are summarized in Figure 2.5. Low concentration of NHx generally accelerates development. At extreme concentration, NHx can be directly toxic, resulting in etching of tissue. At lower concentrations, damage at cellular levels may occur as the detoxification mechanisms, such as pH buffering capacity, are overloaded.

It has been demonstrated that excessive NHx in landfill leachate would hinder germination and seedling growth of grasses and trees, including *Brassica chinensis* and

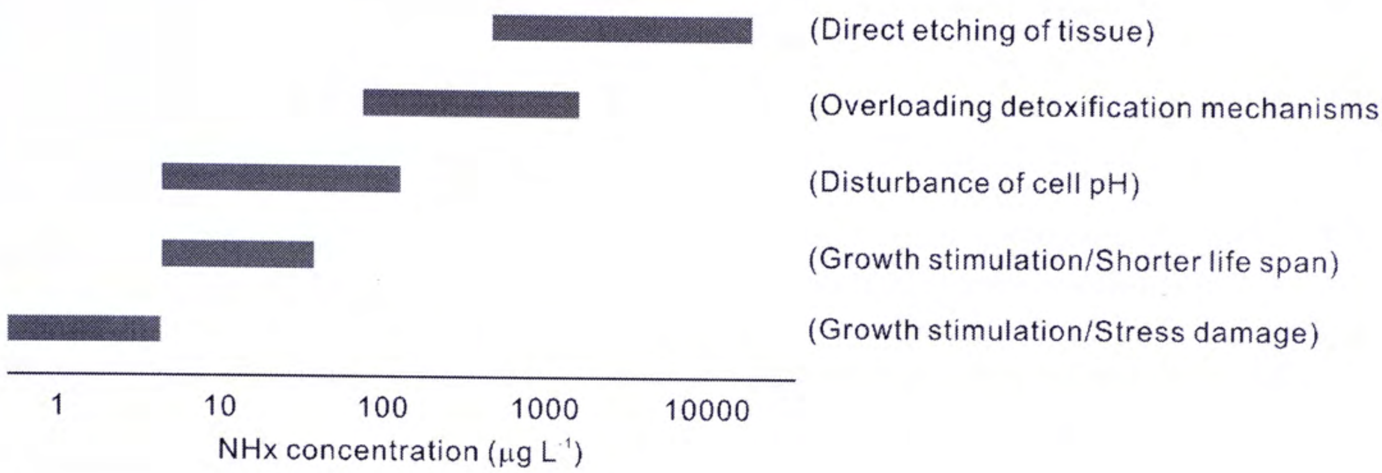


Figure 2.5 An indication of the nature and intensity of the effects of NHx on plants at different concentration ranges (adapted from Dueck and Van der Eerden, 2000).

Lolium perenne (Leung, 1985). O'Brien and Barker (1996) also demonstrated that the inhibitory effects on germination and root growth of *Lolium perenne* were associated with the content of NHx in compost extract. Similar results were reported in a phytotoxicity test of manure extract using seeds of *Brassica parachinensis* (Wong and Lau, 1983).

Na is one of the prevalent ions in landfill leachate. Besides damage to soil structure, many agronomic plant species are seriously injured by a high Na:Ca ratio in the substrate. Addition of Na without the concomitant increase in Ca has been shown to cause specific damage to cell membranes (Cramer *et al.*, 1985).

Organic acids originating from waste degradation were suggested to inhibit germination and root growth (Pascal *et al.*, 1997; Ozores-Hampton *et al.*, 1999). However, they were less likely to be a major cause of leachate phytoxicity from the landfill studies. Methanogenic conditions had been established in these landfills, which were indicated by the alkaline leachates with high NHx content. Acids formed in anaerobic degradation were assimilated for methanogensis.

The results of germination tests provided an overall picture about the phytotoxicity level of landfill leachate. However, owing to the complexity of leachate composition, it is difficult to suggest, with certainty, the major toxicant(s)/factor(s) that contribute to the phytotoxicity. A toxicity identification evaluation (TIE) technique may help to answer the question. Although the TIE protocol (USEPA, 1993) was originally developed for toxicity assay using aquatic organisms, germination tests can be incorporated into the TIE procedure to identify and the major toxicant. Compared with test protocols using mature plant, germination/root elongation tests are more suitable for the TIE procedure as it requires only a small amount of pretreated sample.

2.4.2.4 Comparison between species

Brassica chinensis is not a species suggested by the standard methods since it is less common in the western societies. There was no significant difference ($P > 0.05$) between the EC50s determined for the two plants, implying that *Brassica chinensis* and *Lolium perenne* were equally sensitivive to the toxicity of leachate. The ranges of CI95s determined by *Brassica chinensis* were even smaller, indicating the smaller variation between replicates. With regard to the efficiency of the test, *Brassica chinensis* may be more suitable for phytotoxicity assay. It has a radicle of larger diameter, which is easier to handle and is less susceptible to breakage during root length measurement. Moreover, if root length is determined by imagery analysis thicker root may facilitate the root identification and measurement.

2.5 Conclusions

When rainwater infiltrates through a landfill, it picks up organic and inorganic substances in the decomposing wastes to form leachate. The variation in leachate composition is generally attributed to differences in age, climate and waste composition. Although leachate can provide a considerable amount of macronutrients such as N and K, undiluted leachate is not recommended to be used as irrigation water due to high salinity and sodicity.

This study evaluated the phytotoxicity of 5 leachate samples using seed germination/root elongation tests. Their EC50 ranged from 4% (WENT leachate) to 30% (MYTC leachate) and in general decreased with the strength of leachate samples. Some dose response relationships showed growth stimulation in low doses, which could be attributed to hormesis and the presence of some nutrient constituents in the leachates. The seeds of *Brassica chinensis* and *Lolium perenne* were equally sensitive to leachate phytotoxicity. In the next experiment, they would be used to evaluate the toxicity of two other leachate samples. Results of germination tests should be incorporated into the leachate irrigation practice to safeguard the recipient plants.

Chapter 3 Leachate irrigation: Effects on plant performance and soil properties

3.1 Introduction

On degraded sites, there is usually substantial deficiency of macronutrients, especially N, due to their removal in aboveground biomass, surface litter and topsoil. Hence for land restoration, addition of very large amounts of nitrogenous fertilizer is usually be required (Bradshaw, 1983). Municipal landfill leachate has been suggested to be used as fertilizer because of its high N content. However, experimental studies on the use of landfill leachate for plant growth have reported both positive and detrimental effects. Bramryd (1988) obtained an increase in the biomass productivity of *Dactylis glomerata*, *Salix viminalis* and *Salix aquatica* irrigated with leachates. Liang *et al.* (1999) suggested the use of landfill leachate as irrigation water in dry seasons, in which leachate enhanced the growth, the survival and the stomatal conductance of *Acacia confusa, Leucaena leuocephala* and *Eucalyptus torelliana.* In contrast, irrigation with landfill leachate may lead to reduced yield and poor survival rate (Menser, 1981; Menser *et al.,* 1983).

Contradictory results are not only observed between experiments, but also between plant species within a study. Cureton *et al.* (1991) reported significantly higher growth in *Phalaris arundinacea*, *Salix babylonica* and *Populus nigra* subjected to leachate application, but phytotoxicity symptoms such as brown leaves and necrotic spots were observed in poplar leaves, while chlorophyll degradation or even complete chlorosis were found in willows. These results show that species, leachate source, methods of application and their interactions all had significant influences on the outcome of leachate irrigation.

Moreover, there have been extensive reports on the deterioration in soil quality, such as soil salination (Wong and Leung, 1989; Hernández *et al.*, 1999) and the increase in soil sodicity (Winant, 1981; Chan, 1982) after leachate application. Once occurred, some damages are irreversible. Mitigation measures, if possible, are sometimes costly and time consuming. For example, massive plant mortality and consequent soil erosion may require topsoil replacement and revegetation.

Leachate irrigation can provide a means of wastewater disposal as well as nutrient reuse. However, research on leachate irrigation mainly focused on the use of the soil-plant system for wastewater treatment and disposal. Most of the experiments aimed at investigating and maximizing treatment efficiency. The details of leachate application, for example, the dilution levels, were seldom considered in light of phytotoxicity data of individual leachate samples and this may result in contradictory outcomes. Bowman *et al.* (2002) reported the feasibility of alternating irrigation with leachate and water on turf grass (*Cynodon dactylon* and *Pennisetum clandestinum*) to mitigate the problem of soil salination. Leachate applied at 50% (one leachate irrigation followed by one watering) resulted in yield reduction, but the yield was higher than the control (0%; water only) when the frequency of leachate application was reduced to 20%.

Leachate contains considerable amount of NHx and other nutrients that can be assimilated for plant growth. However, the application rates being tested in the above mentioned studies exceeded the nutrient requirement considerably (especially the N demand). If the aim of leachate irrigation is solely for nutrient supply, there is still flexibility for reducing the application rate to control possible phytotoxicity.

Uncertainty in predicting outcomes, together with the possible harmful effects to vegetation and soil, result in risks associated with using leachate for irrigation. Practitioners of landfill restoration are still reluctant to apply leachate to enhance vegetative growth. It is necessary to develop a more reliable means to evaluate the suitability of landfill leachate for fertigation and determine the method of application.

The composition and phytotoxicity of leachates from some local landfills were assayed in the previous experiment. They carried many soluble compounds originating from the degrading solid wastes. Leachate samples were characterized by high levels of salts, NHx and organics. The concentrations of heavy metals were low compared with those found in the literature. Seed germination/root elongation tests in the previous experiment provided a general picture of the phytotoxicity of landfill leachate.

The present study aimed at using leachate as an alternative to fertilizer. The responses of tree seedlings to leachate irrigation was investigated. An attempt was made to incorporate phytotoxicity data in the design of the leachate irrigation plan to safeguard the plant from mortality or growth inhibition. It was hypothesized that if the recipient plants (tree seedlings) and the germinating seeds were equally sensitive to leachate phytotoxicity, application of leachate at the EC50 level (determined by the germination test) would result in 50% growth inhibition or deterioration in other health indices such as chlorophyll fluorescence. Tissue analysis and biological responses of the test plants (growth, survival, chlorophyll fluorescence, visible symptoms of mineral disorder) were employed, to detect and evaluate the effects of leachate irrigation. Soils after leachate treatments were also analyzed to evaluate the effectiveness of leachate in improving the fertility of the receiving soil and the sustainability of revegetation.

3.2 Materials and methods

Leachate from the PPV Landfill (closed landfill) and the WENT Landfill (operating landfill) were collected in March 2002. Leachates were assayed for their phytotoxicity using seed germination/root elongation tests. Tree seedlings of 12 species were tested with leachate irrigation at the EC50 level determined by the germination test to assess the differences in their response and sensitivity to landfill leachates. The mineral contents of soil and foliage tissue were determined at harvest.

3.2.1 Leachate sampling and analysis

Leachate samples were collected from the leachate extraction well at the PPV Landfill, and the inflow pipe of leachate treatment plant at the WENT Landfill. The samples were stored in air-tight 10-L PVC carboys. They were transported to the laboratory and stored at 4°C. Chemical analysis and phytotoxicity tests were conducted within 24 hours after collection using methods as described in Chapter 2.

3.2.2 Leachate irrigation experiment

Twelve tree species, which included commonly used exotics and less commonly planted natives, were selected for leachate irrigation. Two N-fixing species were included because of their importance in the amelioration of degraded soils as destined for revegetation. Tree seedlings with the height of 20 - 30 cm (1 year old) were purchased from local tree nurseries. They were then transplanted to pots of 19 cm i.d. and 18 cm in height. The soil used was a decomposed granite (DG) collected from the Lam Tei Quarry, Tuen Mun, which was passed through a 5 mm mesh sieve to remove large particles before use.

After acclimation for one month, the tree seedlings were irrigated with leachate diluted with tap water to their respective EC50 levels for a period of 90 days. Each pot was surface irrigated with 12 mm of diluted leachate three times a week, with the same amount of tap water for the control. The volume of irrigation was equivalent to the average rainfall of Hong Kong. Excess moisture was allowed to drain from the bottom of pots. Each treatment had five replicates and the pots were arranged in randomized blocks in a greenhouse (Plate 3.1).

The growth and health of the plants were monitored during the experimental period. Height, basal diameter and standing leaf number were measured every 4 weeks. Foliage biomass, as well as the mineral nutrient contents of soil and foliage tissue were determined at harvest. Chlorophyll fluorescence was determined every 2 weeks using a Plant Efficiency Analyzer (PEA) (Hansatech, England). Additionally, the number of leaves and some visible vegetative effects, such as chlorosis,

Plate 3.1 Tree seedling in 19-cm pots arranged in randomized blocks in a greenhouse. The photo was taken on Day 90 just before harvesting.

browning leaf edges and turgor loss, were observed during the course of leachate application.

3.2.3 Soil and plant analysis

3.2.3.1 Soil sampling and preparation

The soil in pots was sampled by inserting a stainless steel soil core (4 cm i.d.) vertically into the soil at the end of the experiment. Soil in the pots of *Acacia auriculiformis* and *Casuarina equisetifolia*, which are N-fixing, were sampled and analyzed separately from that of non-N-fixing species. Four pots were randomly selected from each treatment. Samples were air-dried for 14 days and passed through a 2 mm sieve before texture determination and chemical analysis.

3.2.3.2 Soil texture

Soil texture was determined by the Bouyoucos hydrometer method which measures the decrease in density of a suspension as soil particles settle (Allen, 1989; Grimshaw, 1989). Air-dried soil was dispersed with 5% sodium hexametaphosphate (Calgon solution) and water. The hydrometer readings were taken at 4 min 48 seconds (for silt and clay contents) and 5 hours (for clay content). The sand, silt and clay contents were expressed as percentages by weight and textural class was determined following the classification of the International Society of Soil Science.

3.2.3.3 pH and electrical conductivity

Soil samples were extracted with Milli Q water at a ratio of 1:2.5 w/v and shaken at 150 rpm for 1 h. The pH and electrical conductivity of the settled suspension were measured with a pH and conductivity meter.

3.2.3.4 Organic carbon

Preweighed samples were analyzed by the IR-combustion method using a Total Organic Carbon Analyzer (TOC5000A, Shimadzu Corporation, Kyoto, Japan) with a Solid Sample Combustion Unit (SSM5000A, Shimadzu Corporation, Kyoto, Japan).

3.2.3.5 Nitrogen

Total N content was determined by a SAN^{Plus} Segmented Flow Auto-analyzer (SAN^{Plus}) after semi micro-Kjeldahl digestion (Skalar, 1995). Extractable NHx-N and NOx-N were measured by SAN^{Plus} after extraction with 1 M KCl at 150 rpm for 1 h (Rowell, 1996).

3.2.3.6 Phosphorus

Total P content was measured by SAN^{Plus} after semi micro- Kjeldahl digestion (Skalar, 1995). Available PO_4^{3-}-P was determined by SAN^{Plus} after extraction with a $(NH_4)_2SO_4$ buffer (pH = 3.0) (Troug's reagent) at 150 rpm for 30 min (Troug, 1930).

3.2.3.7 Chloride

Soil samples were extracted with 0.01 M $Ca(NO_3)_2$ for 30 min (Soil and Plant Analysis Council, 2000), followed by determination using a SAN^{Plus} analyzer.

3.2.3.8 Metals

Total metal contents were determined by a inductively coupled plasma atomic emission spectrometer, after mixed acid (H2SO_4: HNO_3 = 1:5 v/v) digestion at 120°C.

3.2.3.9 Foliage analysis

Foliage was harvested and washed with deionized water. It was then placed in a preweighed paper bag and dried at 65°C to constant weight. Foliage biomass was weighed. The tissue was milled and analyzed for total N and metal contents. The methods of digestion and measurement were the same as those for the soil samples.

3.3 Results and discussion

3.3.1 Leachate

3.3.1.1 Chemical properties

The chemical properties of the two leachate samples are presented in Table 3.1. They possessed some common characteristics of typical landfill leachates. The two samples were slightly alkaline with high COD, TKN and NHx-N contents. They were high in Cl^- and some major cations such as Na, but the concentrations of heavy metals and P were relatively low. Compared with the PPV and WENT leachate assayed in the previous experiment (collected in November 2001), the strength of the leachates collected for this experiment (collected in March 2002) was much lower.

There is a seasonal variation in the composition of landfill leachate. Leachate generated in dry seasons (October to February) usually has a higher strength than samples collected in wet seasons (Wong, 2003). Chu *et al.* (1994) demonstrated that the strength of leachates was negatively correlated with the cumulative rainfall recorded 7 days and 14 days before sampling. Moreover, the large difference in the leachate composition can also be attributed to the weather conditions before sample collection, which influenced the water budget of landfills.

Table 3.1 Properties of leachate samples used for the irrigation experiment.

	Leachate	
	PPV	WENT
pH	7.52	7.96
Electrical conductivity	4.20	17.0
COD	348 ± 5.90	6380 ± 78.4
TOC	35.3 ± 5.35	1970 ± 11.1
Total Kjeldahl nitrogen (TKN)	297 ± 11.1	2130 ± 34.9
Ammoniacal nitrogen (NHx-N)	284 ± 12.3	2210 ± 17.7
Oxidized nitrogen (NOx-N)	< 1.00	< 1.00
Total phosphorus (TP)	10.5 ± 0.05	15.2 ± 0.14
*ortho*phosphate phosphorus (PO_4^{3-}-P)	5.43 ± 0.07	15.0 ± 0.14
Chloride (Cl^-)	297 ± 5.16	3080 ± 21.5
Total metals		
Na	572 ± 3.25	1960 ± 43.9
K	239 ± 2.30	1330 ± 44.5
Ca	54.4 ± 0.60	31.9 ± 0.25
Mg	23.7 ± 0.35	112 ± 1.30
Cd	< 0.001	< 0.001
Cr	< 0.001	0.24 ± 0.00
Cu	< 0.001	0.02 ± 0.00
Fe	4.62 ± 0.00	4.36 ± 0.00
Mn	1.51 ± 0.00	0.02 ± 0.00
Pb	< 0.001	0.15 ± 0.00
Zn	0.10 ± 0.00	0.96 ± 0.00
Soluble metals		
Na	556 ± 5.65	1750 ± 17.6
K	231 ± 2.82	1310 ± 17.9
Ca	23.6 ± 0.25	5.73 ± 0.02
Mg	21.5 ± 2.50	87.2 ± 7.09
Cd	< 0.001	< 0.001
Cr	< 0.001	0.43 ± 0.00
Cu	< 0.001	< 0.001
Fe	0.80 ± 0.00	2.42 ± 0.05
Mn	0.45 ± 0.00	0.01 ± 0.00
Pb	< 0.001	0.08 ± 0.00
Zn	< 0.001	0.55 ± 0.00

All units in mg L^{-1} except for pH (no units) and electrical conductivity (mS cm^{-1}).

March 2002 was much warmer and wetter than usual (Table 3.2). The mean air temperature of 21.5°C was 3.0°C above the normal, the warmest for March. The monthly rainfall of 239 mm was more than three times the normal amount. The cumulative rainfall in the first three months of the year was 268 mm, nearly twice the normal figure for the same period. The total evaporation was 85.5 mm, which was lower than the normal of 92.2 mm (Hong Kong Observatory, 2003 b,c).

The immediate effect of increased water infiltration is flushing of chemical species with high solubility, such as NH_4^+ and Cl^-. There may be a sudden increase in their concentrations. Afterwards, increased water infiltration would dilute the constituents in leachate, leading to a decrease in their strength. Chu *et al.* (1994) demonstrated that the strength of leachates was negatively correlated with the cumulative rainfall recorded 7 days and 14 days before sampling.

3.3.1.2 Phytotoxicity

The phytotoxicity of leachate changed with the composition of the samples. The EC50s of leachates from different landfills ranged from 3% to 46% (v/v) according to the germination test (Table 3.3). The EC50s determined for two plant species, *Brassica chinensis* and *Lolium perenne*, did not differ significantly. The current samples were significantly less toxic than the previous batch of samples. There were also significant differences in the levels of NHx-N and Cl^- in the two batches of leachate samples, suggesting that Cl^- and NH_x were the major constituents contributing to the phytotoxicity. The effects of Cl^- on plants will be discussed in the later sections.

Table 3.2 Meteorological observations in Hong Kong for March 2002 and monthly meteorological normals observed between 1961-1990 (Hong Kong Observatory, 2003 b, c).

Meteorological parameter	Normal value	Value observed in March 2002
Mean air temperature (^{o}C)	18.5	21.5
Rainfall (mm)	66.9	239
Cumulative rainfall (Jan - Mar) (mm)	141	268
Total evaporation (mm)	92.2	85.5

Table 3.3 The EC50s of leachate samples determined by seed germination/root elongation tests.

	EC50 (% v/v) ± CI95	
	Lolium perenne	*Brassica chinensis*
PPV leachate	45.9 ± 1.84	32.8 ± 2.50
WENT leachate	5.52 ± 0.54	2.95 ± 0.81

CI95 is the confidence interval at 95% of EC50 determined by 4 replicates.

3.3.2 Plant responses

Using landfill leachate as a source of nutrients is not practicable if it leads to mass plant mortality or deterioration of soil fertility. The success of leachate irrigation depends on balancing the beneficial effect of nutrient supply to plants, and the detrimental effects of some phytotoxic substances/factors such as high salinity and excessive NHx.

3.3.2.1 Growth

Figure 3.1 shows the percentage growth in plant height after 90 days. In general, the leachate-treated plants had better growth performance than those receiving water alone. Although the strength of the WENT leachate was much higher than that of the PPV leachate, most of the species did not exhibit significant growth differences in the two leachate treatments.

Instead of diluting the leachates arbitrarily, the levels of dilution were determined based on the biological responses of germinating seeds. Each plant received leachate irrigation at their respective EC50 (i.e. the concentration which led to a half of the intensity of the biological response in the control group). The dilutions for the two leachates, in terms of percentage by volume, were different. The higher the strength of raw leachate, the lower is the concentration of leachate applied. Thus, the intensity of the resulting biological response (growth promotion or inhibition) should be similar.

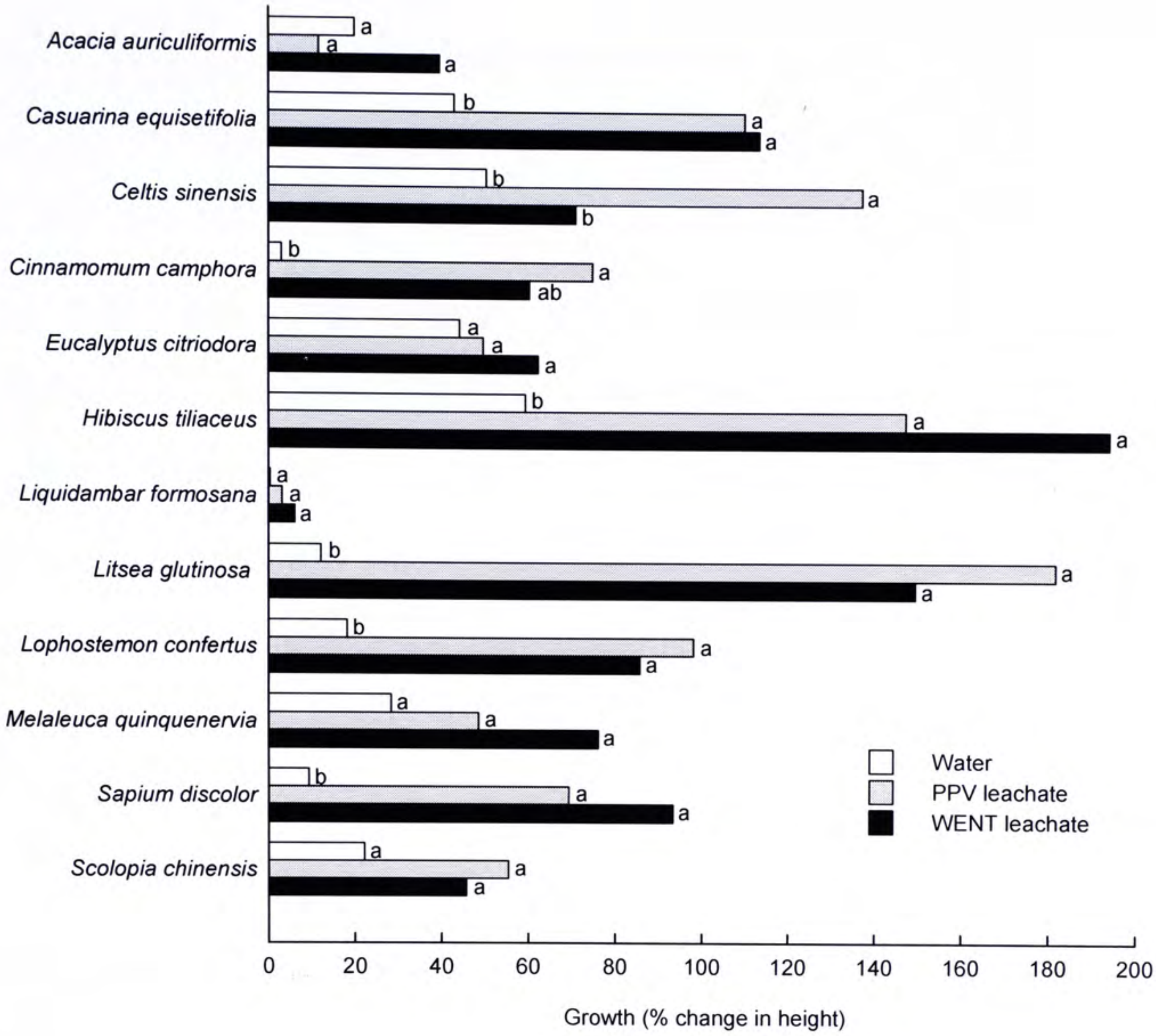


Figure 3.1 Plant growth in height after 90-day irrigation with water or leachate from the PPV and WENT Landfills. When compared within the same species, bars with the same letter are not significantly different at $P > 0.05$ by Tukey's test.

When compared among the 12 species used in this experiment, *Hibiscus tiliaceus* and *Litsea glutinosa* exhibited remarkable growth promotion with leachate irrigation (Plates 3.2 and 3.3); their growth were 3 and 12 times that of the control, respectively. Differences in growth could be attributed to the differential responses to the addition of nutrients. There is ample literature on accelerated tree growth after N application. However, only a few studies addressed the differential responses in different tree species. A classic study of the response of a forest to fertilizer application was that done in New England by Mitchell and Chandler (1939). Tree species were classified as N-demanding, N-tolerant and intermediate. The N-tolerant species grew relatively well in soil with low available N but did not increase growth as much as N-demanding species after fertilizer application. Besides the different responses to N applied with leachate, difference in growth can also be attributed to the tolerance to inhibitory constituents in the leachates. *Hibiscus tiliaceus* is a salt-tolerant plant which inhabits coastal areas such as seashores where substrate salinity is relatively high. It seems to be less susceptible to soil salination as a result of leachate irrigation.

On the other hand, the N-fixing legume *Acacia auriculiformis* was not superior to other non-N-fixing plants in growth under leachate irrigation. The symbiotic N-fixing capability allows acacia to grow well on infertile granitic soil (Corlett, 1999; Chong, 1999). However, it was no longer an advantage when there was ample supply of N from the leachates. Moreover, the high level of NHx, or possibly other constituents in leachate, may be detrimental to symbiotic N fixation. Leachate irrigation resulted in poor nodule development and reduced nitrogenase activity (Chan *et al.*, 1999). In the present study, the root nodules on leachate-treated acacias were black in colour

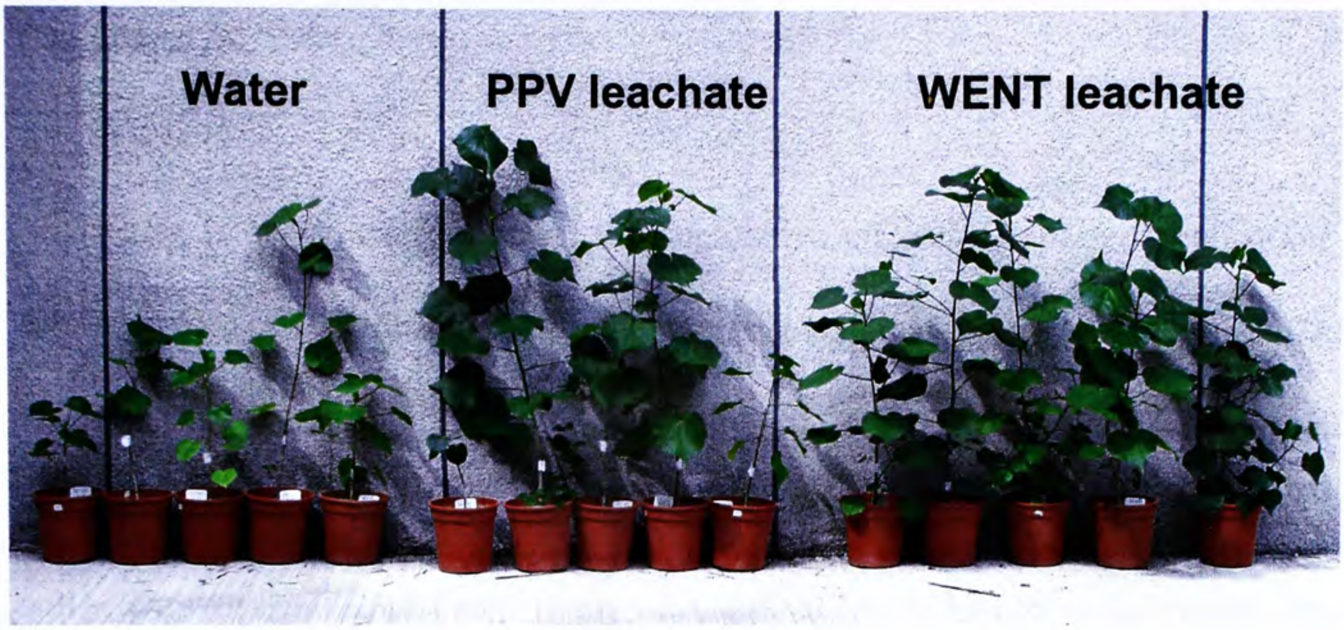


Plate 3.2 *Hibiscus tiliaceus* after being irrigated with water and leachates for 90 days. Compared with the water treatment, the color of foliage was darker in the leachate treatments. The trees that received leachates had denser leaves with larger leaf area. Trees receiving WENT leachate had many newly grown leaves on the lower part of the stem, possibly due to excessive nutrient supply.

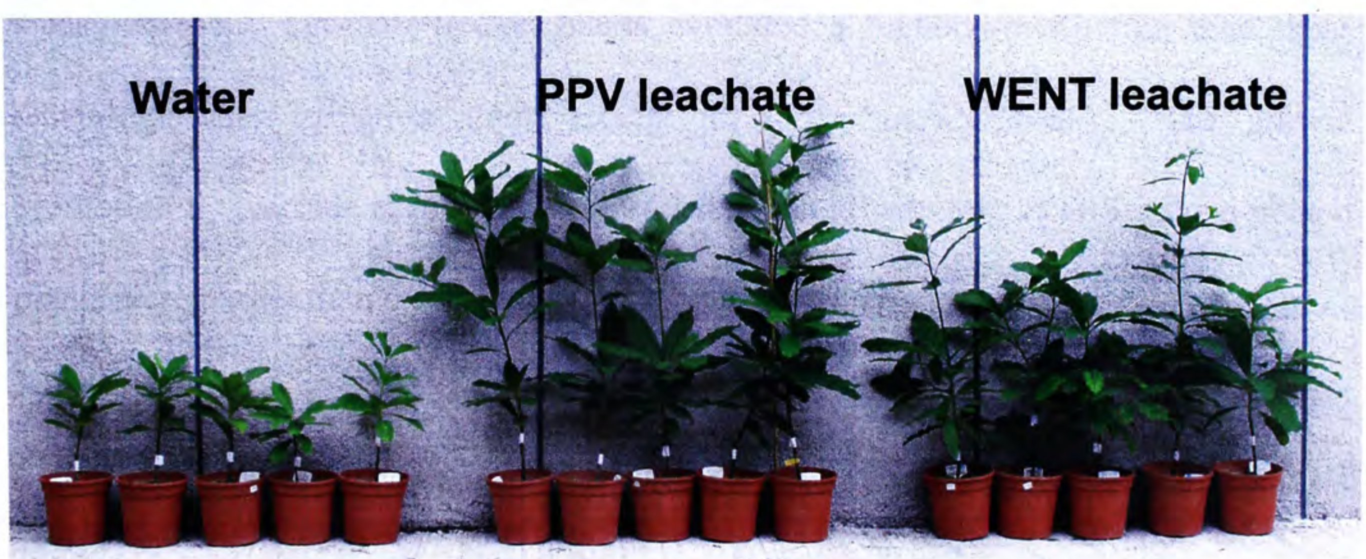


Plate 3.3 *Litsea glutinosa* after irrigated with water and leachates for 90 days. The differences in growth between treatment groups was more evident than *Hibiscus tiliaceus*. The leaf area in leachate-treated plants were much larger, up to 4 - 6 times of those in the control group. Moreover, the leaf colour of leachate treated plants was darker.

(Plates 3.4 and 3.5), which indicates poor nodule formation and low N-fixing activity (Dreyfus *et al.*, 1987).

There is also a concern about the cumulative effects of repeated application of diluted leachate. Plants may benefit from the external supply of nutrients (from leachate) and grow rapidly at the beginning of leachate irrigation. The specific growth rate reduces gradually when the inhibitory substances (if any) build up in the soil. Growth evaluation based solely on the plant height measured at the end of the experiment may fail to detect the increasing inhibitory effects of repeated dose. Monitoring growth rate, rather than height, can solve this limitation. Figure 3.2 presents the percentage change in the plant height during the course of leachate irrigation. The slopes of the lines indicate the growth rate between successive height measurements. Leachate-treated plants exhibited a higher growth rate than those receiving water irrigation alone, except in *Acacia auriculiformis*. The growth promotion by leachate seems to be continued as the growth rate of all leachate-treated plants were higher than the controls throughout the experiment.

Basal diameters of tree seedlings (Table 3.4) showed a similar trend. Leachate treated plants grew equally well or better than the control treatments. However, the basal diameter was less sensitive in detecting the growth difference between treatments, probably because of a slower change in girth and relatively larger error between measurements. Also the period of leachate irrigation was too short for trees to exhibit differences of statistical significance.

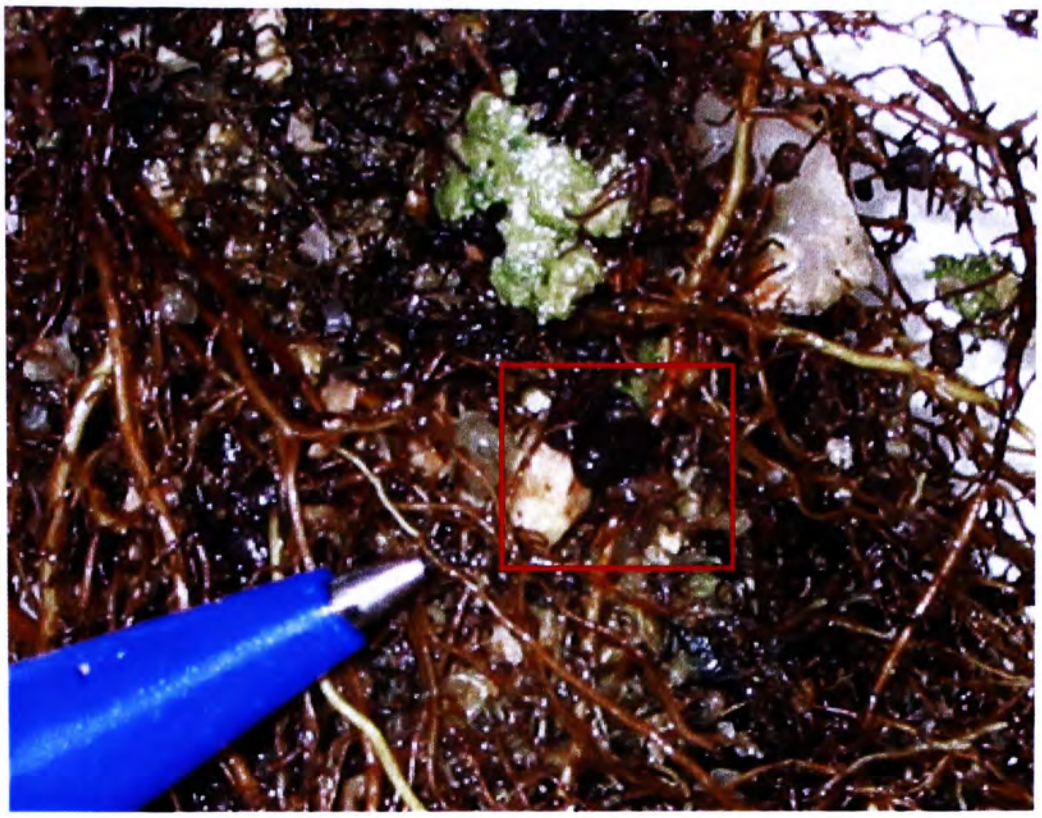


Plate 3.4 A close-up of root nodules (within the red rectangle) in an *Acacia auriculiformas* seedling irrigated with diluted WENT leachate. The density of nodules in leachate-treated plants was lower. The nodules were in a blackish brown colour, which indicates poor nodule formation and low N-fixing activity.

Plate 3.5 Root nodules of an *Acacia auriculiformas* seedling irrigated with water.

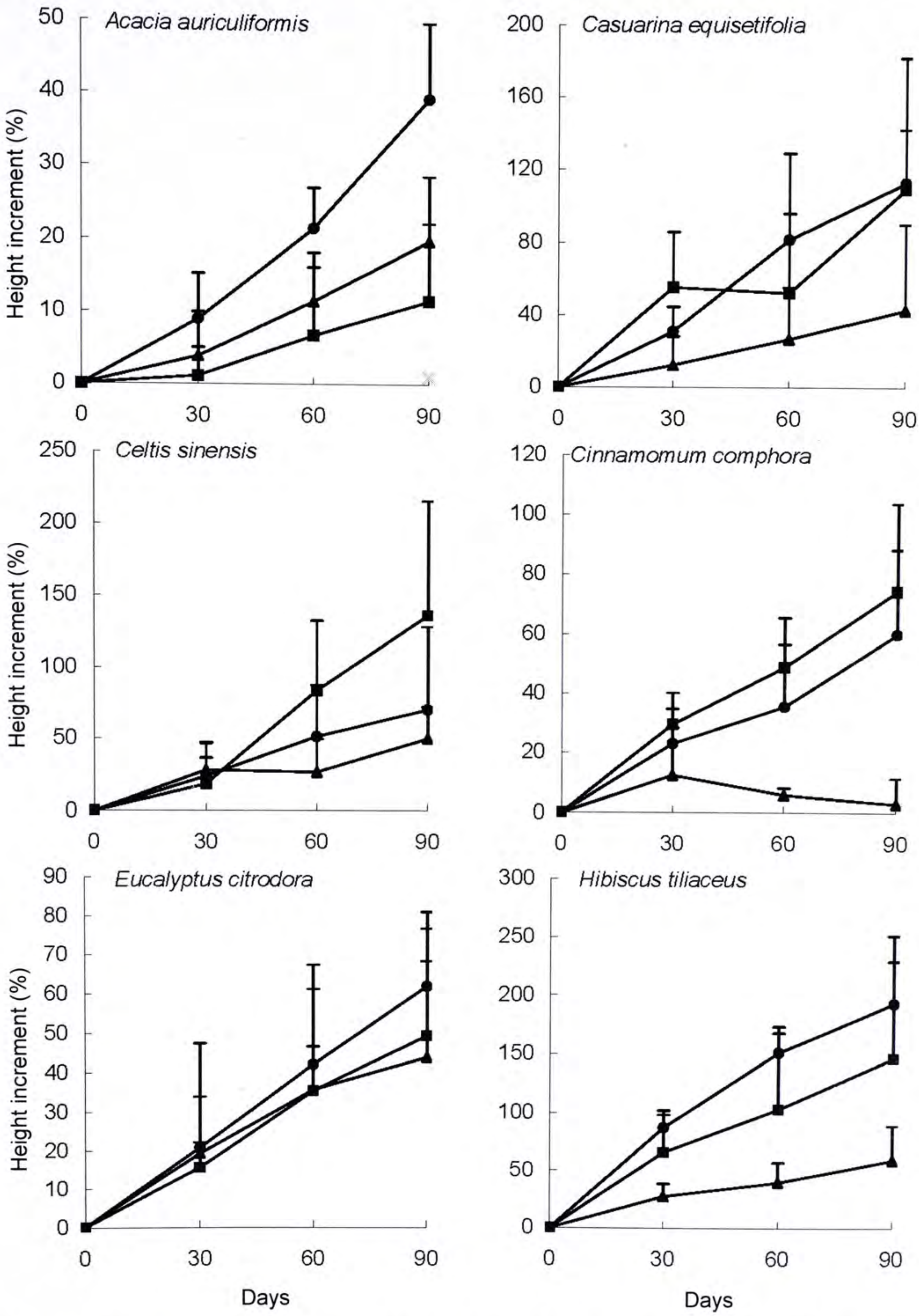


Figure 3.2 Percentage change in height of plants, after irrigation with water (▲) and leachates from PPV (■) and WENT (●) Landfills. Error bars show the standard deviation of 5 replicates.

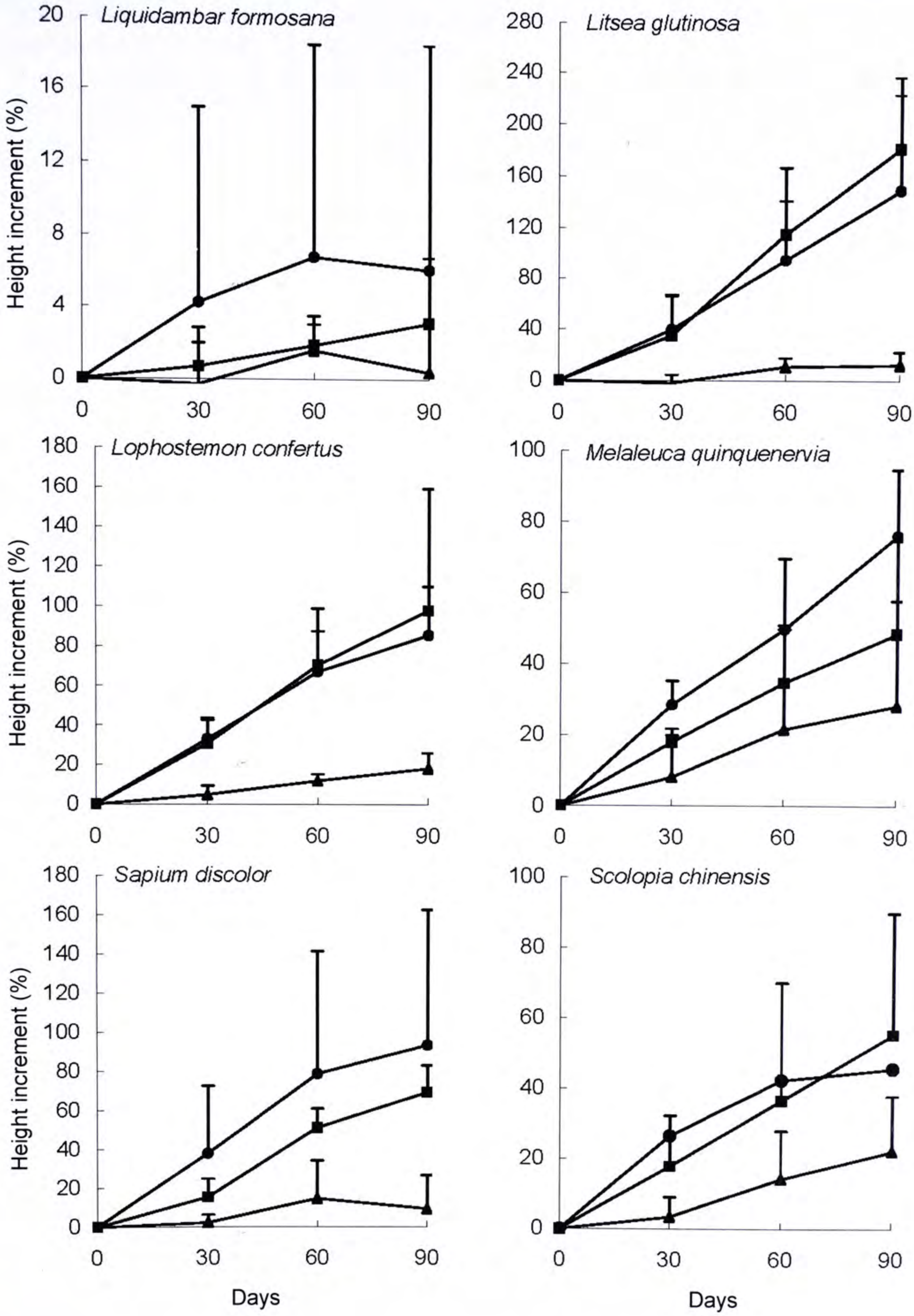


Figure 3.2 (cont'd) Percentage change in height of plants, after irrigation with water (▲) and leachates from PPV (■) and WENT (●) Landfills. Error bars show the standard deviation of 5 replicates.

Table 3.4 Plant growth in basal diameter after 90-day irrigation with water and leachate from PPV and WENT Landfills.

	Percentage change (%)		
Species	Water	PPV leachate	WENT leachate
Acacia auriculiformis	28.4 ± 18.8 a	33.3 ± 10.8 a	18.3 ± 9.87 a
Casuarina equisetifolia	12.1 ± 9.58 a	6.54 ± 27.9 a	17.4 ± 18.7 a
Celtis sinensis	24.0 ± 27.3 a	30.3 ± 24.0 a	10.4 ± 12.4 a
Cinnamomum camphora	-2.82 ± 6.85 b	26.1 ± 8.77 a	28.3 ± 32.5 a
Eucalyptus citriodora	16.0 ± 4.73 a	16.7 ± 26.6 a	27.2 ± 11.5 a
Hibiscus tiliaceus	16.4 ± 9.29 b	29.9 ± 15.5 b	75.7 ± 15.9 a
Liquidambar formosana	-2.23 ± 8.43 a	1.37 ± 10.5 a	3.86 ± 14.9 a
Litsea glutinosa	-6.85 ± 7.40 a	15.8 ± 11.8 a	16.0 ± 11.6 a
Lophostemon confertus	-3.53 ± 14.4 a	13.5 ± 13.9 a	-3.41 ± 15.1 a
Melaleuca quinquenervia	29.1 ± 34.7 a	38.2 ± 19.2 a	31.2 ± 13.2 a
Sapium discolor	3.06 ± 8.15 b	21.0 ± 8.50 ab	30.7 ± 17.8 a
Scolopia chinensis	3.21 ± 7.99 a	8.15 ± 14.4 a	6.09 ± 13.7 a

When compared within species, means followed by the same letters are not significantly different at $P > 0.05$ by Tukey's test.

Leachate treatments not only led to growth in height, but also resulted in biomass increase. The foliage biomass of leachate-treated plants was significantly higher than that in the controls (Table 3.5). Increase in foliage biomass can be attributed to larger leaf area and more/denser leaves on plants (Plates 3.2 and 3.3). The standing leaf number and leaf area of leachate-treated plants were remarkably larger, probably as a consequence of increased nutrient supply. Increased vegetative growth may not be beneficial in some circumstances. For example, delicate young seedlings with very large and dense leaves may easily lodge with wind drift. Moreover, leaves are susceptible to damage as they rub against each other in strong wind. Excessive application of N would also delay crop maturity and the plants are more susceptible to diseases and pests (Brady, 1990; Marschner, 1995). The ecological consequences of excessive N supply will be discussed in a later section.

3.3.2.2 Plant survival and health

Plants respond quickly to the increase in soil salinity, especially when Na^+ and Cl^- are the contributing factors. Irrigation with saline water results in chlorosis, leaf burn and massive leaf fall. Some of these injuries are irreversible. In more severe case; plants can wither in a few days. All tree seedlings tested in this experiment survived until harvest. Their health was monitored qualitatively (visible symptoms) and quantitatively (standing leaf number and chlorophyll fluorescence) to detect the appearance of stress responses.

When compared with the data collected before leachate application, no massive leaf fall (Figure 3.3) or significant changes in the fluorescence parameters (data not

Table 3.5 Foliage biomass harvested after 90-day irrigation with water and leachate from PPV and WENT Landfills.

Species	Foliage biomass (g)		
	Water	PPV leachate	WENT leachate
Acacia auriculiformis	13.1 ± 2.64 a	12.4 ± 1.50 a	12.5 ± 2.65 a
Casuarina equisetifolia	12.5 ± 5.62 a	13.7 ± 4.23 a	15.5 ± 4.95 a
Celtis sinensis	6.17 ± 6.53 a	8.06 ± 1.56 a	6.59 ± 0.46 a
Cinnamomum camphora	8.35 ± 1.14 b	19.7 ± 3.94 a	22.1 ± 3.15 a
Eucalyptus citriodora	9.96 ± 0.79 a	12.5 ± 2.04 a	12.5 ± 0.89 a
Hibiscus tiliaceus	9.16 ± 1.32 b	12.9 ± 6.29 b	20.1 ± 3.40 a
Liquidambar formosana	6.70 ± 0.41 a	7.63 ± 1.33 a	8.14 ± 1.23 a
Litsea glutinosa	9.00 ± 1.35 b	19.7 ± 1.30 a	16.7 ± 13.5 a
Lophostemon confertus	10.0 ± 1.20 b	15.2 ± 2.62 a	11.5 ± 2.72 ab
Melaleuca quinquenervia	8.31 ± 1.65 a	10.5 ± 1.90 a	11.2 ± 2.10 a
Sapium discolor	6.34 ± 0.83 b	13.3 ± 2.32 a	13.8 ± 5.10 a
Scolopia chinensis	7.45 ± 0.70 a	7.92 ± 1.46 a	8.79 ± 0.34 a

When compared within species, means followed by the same letters are not significantly different at $P > 0.05$ by Tukey's test.

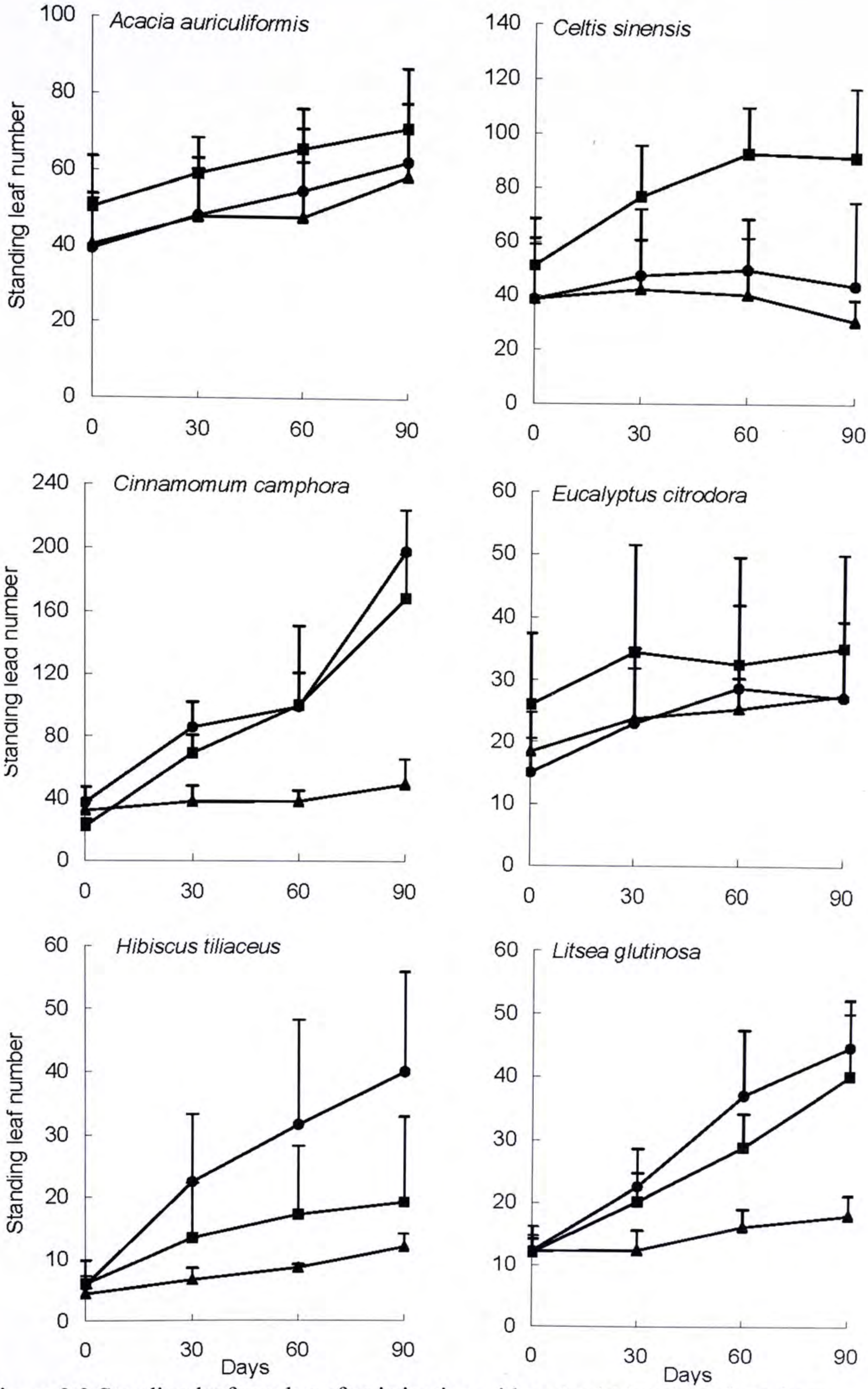


Figure 3.3 Standing leaf number after irrigation with water (▲) and leachate from PPV (■) and WENT (●) Landfills. Error bars show the standard deviation of 5 replicates.

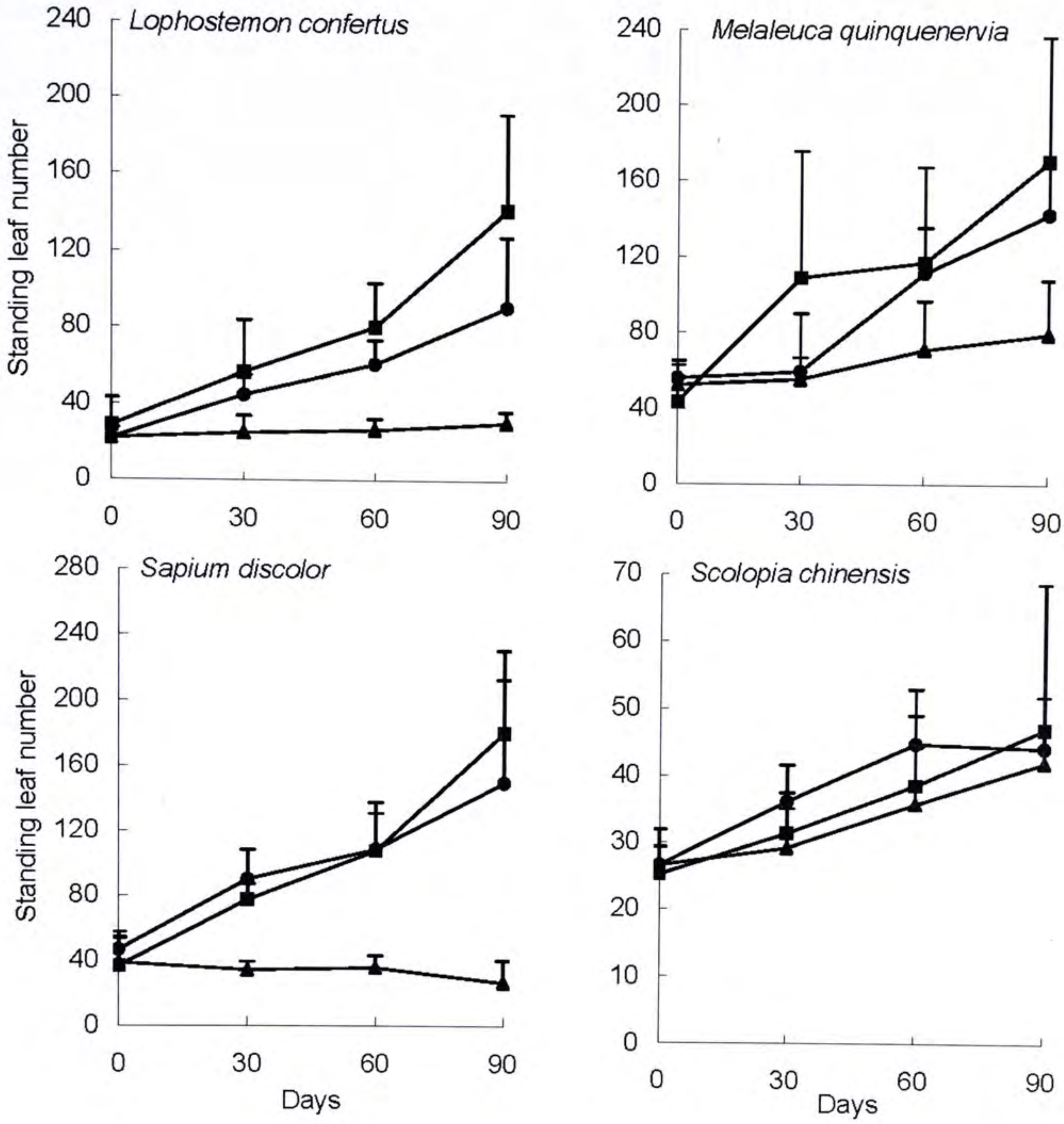


Figure 3.3 (cont'd) Standing leaf number after irrigation with water (▲) and leachate from PPV (■) and WENT (●) Landfills. Error bars show the standard deviation of 5 replicates.

shown) were observed during the course of leachate irrigation. The environmental stress imposed by the application of leachate was well below the tolerance limits of the plants.

Leachate irrigation also brought about some changes in the appearance of plants. Leachate-treated plants had a dark green color, which can be attributed to the supply of soil N. Waskom *et al.* (1997) reported the effect of soil N on the absorbance of leaves. Plants with adequate supply of N had a darker leaf color than those growing in N-deficient soil. However, salinity stress could also result in similar symptoms. Plant growth was stunted, the leaves were dull green in colour and the leaf margins were curled towards the lower surface of leaves (Rusell, 1973). Leung (1985) reported some of these symptoms in plants (*Brassica chinensis* and *B. parachinensis*) treated with 20 and 40% leachate. However, it was very difficult to distinguish between the two possible causes as excessive N supply and slight to moderate increase in soil salinity would lead to similar symptoms.

Plant symptoms may be a useful indication of the nature of mineral disorders and other environmental stresses. Most nutrient deficiencies and elemental toxicities impair plant metabolism in a characteristic way which is manifested in visible symptoms on plants. Taking N as an example, deficiency not only stunts plant growth, but also results in pale green leaves, sometimes with reddening on the underside of leaves. On the other hand, toxicity of excessive NH_4^+ may cause blackening around tips and edges of leaves (Grundon *et al.*, 1997).

Standing leaf number and chlorophyll fluorescence provide rapid and non-destructive means to reflect acute stresses on plants. Stephens *et al.* (2000) reported that *Salix viminalis* progressively lost their leaves after dosing with Cl^- at the rate of 200 mM (equivalent to 7.1 mg L^{-1}). Chlorophyll fluorescence can probe changes in the photosynthetic apparatus before visible injury appears. A decrease in dark-adapted F_v/F_m and increase in F_o indicate the disturbance in response to water stress (Epron *et al.*, 1992), nutritional stress and the exposure to phytotoxic chemicals (Kapustak, 1994; Gemel *et al.*, 1997).

However, reliance on plant symptoms and the change in photosynthetic efficiency has several limitations. For example, symptoms generally develop only in cases of severe disorder. Moderate or transient deficiency (or toxicity) may significantly depress plant growth without producing any symptom that can be diagnosed. Moreover, chlorophyll parameters and plant symptoms may not be specific. Under certain conditions, different nutrient disorders can produce rather similar symptoms (Grundon *et al.*, 1997). Practitioners need to be able to distinguish between symptoms caused by nutrient disorder and those caused by other environmental stressors. Confirmation by other methods such as plant and soil analysis is essential.

3.3.2.3 Tissue contents

Instead of analyzing the mineral content of the whole plant, specific parts of plants are usually more appropriate for the diagnosis of mineral disorders. For example, older plant parts better reflect the nutrient status of the phloem mobile elements, such as N, P and K; while young/growing parts are better for phloem immobile elements (e.g.

Ca, Mn, B) (Smith and Loneragan, 1997). This study focused on the foliar contents. The youngest matured leaf often represents a suitable compromise for most elements where specific guidelines are lacking; it has the advantage that interpretation can be made for a wide range of elements in a single plant part (Smith and Loneragan, 1997).

Table 3.6 presents the foliar N contents of tree seedlings at harvest. Plants treated with WENT leachate had higher foliar N contents than those with water irrigation alone when compared within species. In some species, such as *Litsea glutinosea* and *Lophostemon confertus*, the foliar N contents even doubled those of the controls. NHx-N in leachates was rapidly assimilated and incorporated into the vegetative tissue. Unlike soil NHx and NO_3^-, which are susceptible to leaching loss, the N in biomass would become a part of the long term nutrient reserve of the ecosystem. It would be released in mineralization of litter and provide N for later plant growth. Even if leachate irrigation is terminated, newly invaded species would also benefit by the increased N reserve at the restored site.

Chemistry of foliage tissue reflects the plant uptake of nutrients and pollutants. There is a concern that plant uptake of pollutants, which are originally immobilized in soil, would bring those pollutants back to the ecosystem and accumulate along the food chain. It has been demonstrated that the application of landfill leachate in general increased the foliar content of N (Gordon *et al.*, 1989) as well as other elements such as Mg and Ca (Hernández *et al.*, 1999). However, the foliar contents of metals in leachate-treated plants were not significantly different ($P > 0.05$) to the control group (Table 3.7). The two leachate contained only low concentration of heavy metals.

Table 3.6 Foliar N content, after 90-day irrigation with water and leachate from PPV and WENT Landfills.

Species	Foliar N content (% w/w)		
	Water	PPV leachate	WENT leachate
Acacia auriculiformis	1.53 ± 0.22 b	2.07 ± 0.22 b	2.82 ± 0.49 a
Casuarina equisetifolia	1.37 ± 0.50 b	1.84 ± 0.50 ab	2.06 ± 0.17 a
Celtis sinensis	1.95 ± 0.29 b	2.43 ± 0.29 ab	2.68 ± 0.39 a
Cinnamomum camphora	1.08 ± 0.21 b	1.87 ± 0.21 a	2.13 ± 0.12 a
Eucalyptus citriodora	0.84 ± 0.11 b	1.19 ± 0.11 ab	1.68 ± 0.31 a
Hibiscus tiliaceus	1.40 ± 0.26 b	2.10 ± 0.26 a	2.12 ± 0.24 a
Liquidambar formosana	1.38 ± 0.26 b	1.56 ± 0.26 ab	2.15 ± 0.09 a
Litsea glutinosa	1.23 ± 0.17 b	2.28 ± 0.17 b	2.68 ± 0.40 a
Lophostemon confertus	0.91 ± 0.10 c	2.50 ± 0.11 b	1.86 ± 0.23 a
Melaleuca quinquenervia	1.12 ± 0.12 b	1.44 ± 0.12 b	2.15 ± 0.25 a
Sapium discolor	1.69 ± 0.20 b	2.49 ± 0.20 a	2.69 ± 0.27 a
Scolopia chinensis	0.92 ± 0.11 b	1.72 ± 0.11 a	2.09 ± 0.26 a

When compared within species, means followed by the same letters are not significantly different at $P > 0.05$ by Tukey's test.

Table 3.7 Foliar metal contents after 90-day irrigation with water and leachate from PPV and WENT Landfills.

Species	Treatment	Foliar metal contents (% w/w)			
		Na	K	Mg	Ca
Acacia auriculiformis	Water	0.13 ± 0.15	0.68 ± 0.11	0.12 ± 0.00	0.83 ± 0.05
	PPV leachate	0.23 ± 0.00	0.73 ± 0.07	0.08 ± 0.02	0.79 ± 0.16
	WENT leachate	0.31 ± 0.02	0.56 ± 0.06	0.10 ± 0.01	0.76 ± 0.13
Casuarina equisetifolia	Water	0.10 ± 0.00	0.97 ± 0.08	0.14 ± 0.01	0.86 ± 0.08
	PPV leachate	0.43 ± 0.01	1.05 ± 0.23	0.14 ± 0.02	0.86 ± 0.08
	WENT leachate	0.24 ± 0.01	1.05 ± 0.21	0.10 ± 0.02	0.85 ± 0.08
Celtis sinensis	Water	0.09 ± 0.10	0.65 ± 0.17	0.27 ± 0.03	0.79 ± 0.08
	PPV leachate	0.07 ± 0.02	0.73 ± 0.11	0.18 ± 0.04	0.85 ± 0.08
	WENT leachate	0.12 ± 0.05	0.68 ± 0.10	0.23 ± 0.01	0.85 ± 0.08
Cinnamomum camphora	Water	0.07 ± 0.03	0.61 ± 0.04	0.07 ± 0.08	0.84 ± 0.08
	PPV leachate	0.06 ± 0.01	0.75 ± 0.03	0.10 ± 0.02	0.71 ± 0.12
	WENT leachate	0.11 ± 0.00	0.86 ± 0.01	0.13 ± 0.02	0.73 ± 0.01
Eucalyptus citriodora	Water	1.22 ± 0.13	0.07 ± 0.01	0.17 ± 0.02	0.84 ± 0.08
	PPV leachate	1.25 ± 0.21	0.06 ± 0.00	0.17 ± 0.01	0.72 ± 0.03
	WENT leachate	1.36 ± 0.02	0.13 ± 0.12	0.16 ± 0.02	0.66 ± 0.01
Hibiscus tiliaceus	Water	0.09 ± 0.02	1.66 ± 0.28	0.45 ± 0.15	0.85 ± 0.08
	PPV leachate	0.21 ± 0.12	1.72 ± 0.16	0.55 ± 0.25	0.85 ± 0.08
	WENT leachate	0.22 ± 0.09	1.84 ± 0.11	0.25 ± 0.02	0.84 ± 0.08

Table 3.7 (cont'd) Foliar metal contents after 90-day irrigation with water and leachate from PPV and WENT Landfills.

Species	Treatment	Foliar metal contents (% w/w)			
		Na	K	Mg	Ca
Liquidambar formosana	Water	0.13 ± 0.01	1.15 ± 0.42	0.13 ± 0.02	0.85 ± 0.08
	PPV leachate	0.06 ± 0.01	0.75 ± 0.03	0.10 ± 0.02	0.71 ± 0.12
	WENT leachate	0.15 ± 0.04	1.14 ± 0.10	0.10 ± 0.00	0.66 ± 0.15
Lophostemon confertus	Water	0.08 ± 0.00	1.05 ± 0.01	0.23 ± 0.07	0.85 ± 0.08
	PPV leachate	0.52 ± 0.58	1.23 ± 0.16	0.22 ± 0.05	0.85 ± 0.08
	WENT leachate	0.09 ± 0.03	1.18 ± 0.20	0.14 ± 0.12	0.83 ± 0.11
Litsea glutinosa	Water	0.06 ± 0.02	1.27 ± 0.25	0.09 ± 0.03	0.67 ± 0.16
	PPV leachate	0.07 ± 0.02	1.07 ± 0.13	0.07 ± 0.01	0.57 ± 0.02
	WENT leachate	0.38 ± 0.46	0.94 ± 0.03	0.04 ± 0.01	0.42 ± 0.01
Melaleuca quinquenervia	Water	0.14 ± 0.01	0.67 ± 0.07	0.22 ± 0.01	0.85 ± 0.08
	PPV leachate	0.19 ± 0.16	0.98 ± 0.25	0.24 ± 0.02	0.85 ± 0.08
	WENT leachate	0.37 ± 0.03	0.67 ± 0.03	0.21 ± 0.01	0.85 ± 0.08
Sapium discolor	Water	0.09 ± 0.00	1.28 ± 0.35	0.14 ± 0.12	0.74 ± 0.07
	PPV leachate	0.07 ± 0.01	1.04 ± 0.01	0.09 ± 0.11	0.77 ± 0.05
	WENT leachate	0.08 ± 0.04	1.09 ± 0.02	0.07 ± 0.07	0.59 ± 0.08
Scolopia chinensis	Water	0.41 ± 0.40	1.61 ± 0.14	0.16 ± 0.07	0.80 ± 0.05
	PPV leachate	0.10 ± 0.01	1.38 ± 0.21	0.13 ± 0.03	0.69 ± 0.06
	WENT leachate	0.13 ± 0.01	1.27 ± 0.12	0.15 ± 0.03	0.77 ± 0.02

Table 3.7 (cont'd) Foliar metal contents after 90-day irrigation with water and leachate from PPV and WENT Landfills.

Species	Treatment	Foliar metal contents (mg kg^{-1})					
		Cd	Cr	Cu	Fe	Pb	Zn
Acacia auriculiformis	Water	1.66 ± 0.69	5.41 ± 0.12	6.61 ± 1.01	30.1 ± 42.6	0.03 ± 0.00	0.10 ± 0.10
	PPV leachate	0.96 ± 0.48	3.16 ± 2.46	3.63 ± 3.15	67.3 ± 33.3	0.02 ± 0.01	0.03 ± 0.01
	WENT leachate	1.51 ± 0.58	4.53 ± 1.66	43.2 ± 52.0	50.2 ± 41.2	0.02 ± 0.01	0.03 ± 0.01
Casuarina equisetifolia	Water	1.29 ± 0.44	3.80 ± 3.18	9.81 ± 4.86	34.7 ± 34.5	0.07 ± 0.07	0.05 ± 0.01
	PPV leachate	1.68 ± 0.57	8.74 ± 1.64	10.4 ± 2.56	93.7 ± 1.40	0.03 ± 0.00	0.07 ± 0.01
	WENT leachate	0.76 ± 0.02	3.00 ± 0.18	9.90 ± 4.53	64.1 ± 9.83	0.01 ± 0.01	0.05 ± 0.02
Celtis sinensis	Water	1.70 ± 0.42	10.5 ± 2.82	12.5 ± 3.03	113 ± 18.5	0.03 ± 0.01	0.05 ± 0.08
	PPV leachate	1.44 ± 0.44	11.9 ± 6.47	13.0 ± 3.82	151 ± 91.6	0.03 ± 0.00	0.04 ± 0.00
	WENT leachate	1.83 ± 0.78	8.49 ± 2.03	12.6 ± 7.37	84.0 ± 34.2	0.03 ± 0.02	0.09 ± 0.08
Cinnamomum camphora	Water	1.54 ± 0.90	8.66 ± 4.54	8.20 ± 2.97	80.2 ± 77.6	0.04 ± 0.01	0.05 ± 0.01
	PPV leachate	0.68 ± 0.71	13.7 ± 8.96	4.50 ± 4.91	28.3 ± 12.8	0.01 ± 0.02	0.05 ± 0.01
	WENT leachate	2.05 ± 0.57	8.66 ± 4.90	12.4 ± 3.41	56.3 ± 45.4	0.03 ± 0.01	0.04 ± 0.00
Eucalyptus citriodora	Water	1.61 ± 0.41	5.23 ± 2.05	6.95 ± 1.24	46.4 ± 58.2	0.02 ± 0.00	0.13 ± 0.05
	PPV leachate	1.46 ± 1.11	3.84 ± 0.27	9.01 ± 2.32	62.8 ± 88.8	0.02 ± 0.00	0.07 ± 0.00
	WENT leachate	2.13 ± 0.35	7.56 ± 3.38	14.6 ± 4.12	29.1 ± 41.1	0.03 ± 0.00	0.07 ± 0.00
Hibiscus tiliaceus	Water	1.99 ± 1.40	15.2 ± 16.1	43.0 ± 38.9	69.1 ± 19.4	0.03 ± 0.02	0.11 ± 0.11
	PPV leachate	1.58 ± 1.17	11.0 ± 15.5	21.3 ± 22.8	124 ± 142	0.03 ± 0.03	0.06 ± 0.03
	WENT leachate	1.80 ± 0.60	10.0 ± 4.30	13.1 ± 2.76	81.4 ± 35.6	0.03 ± 0.00	0.04 ± 0.01

Table 3.7 (cont'd) Foliar metal contents after 90-day irrigation with water and leachate from PPV and WENT Landfills.

Species	Treatment	Foliar metal contents (mg kg^{-1})					
		Cd	Cr	Cu	Fe	Pb	Zn
Liquidambar formosana	Water	1.78 ± 0.99	7.49 ± 4.08	11.5 ± 6.33	96.4 ± 90.8	0.03 ± 0.00	0.06 ± 0.02
	PPV leachate	0.68 ± 0.71	13.7 ± 8.96	4.50 ± 4.91	28.3 ± 12.8	0.01 ± 0.02	0.05 ± 0.01
	WENT leachate	1.16 ± 0.87	6.48 ± 5.98	62.2 ± 59.4	87.8 ± 50.1	0.02 ± 0.01	0.05 ± 0.01
Lophostemon confertus	Water	1.26 ± 0.23	4.60 ± 1.27	6.23 ± 0.42	72.7 ± 51.4	0.02 ± 0.00	0.07 ± 0.00
	PPV leachate	2.06 ± 0.80	13.4 ± 5.67	13.9 ± 1.91	143 ± 22.5	0.04 ± 0.01	0.06 ± 0.00
	WENT leachate	86.9 ± 121	4.96 ± 2.49	8.40 ± 1.20	52.4 ± 6.81	0.02 ± 0.01	0.06 ± 0.02
Litsea glutinosa	Water	1.56 ± 0.16	6.98 ± 2.97	9.35 ± 1.31	315 ± 297	0.03 ± 0.00	0.07 ± 0.02
	PPV leachate	2.08 ± 0.60	6.83 ± 4.70	10.7 ± 1.98	63.7 ± 54.1	0.01 ± 0.01	0.08 ± 0.03
	WENT leachate	0.96 ± 0.94	2.04 ± 2.88	9.30 ± 3.25	46.1 ± 51.9	0.02 ± 0.01	0.06 ± 0.03
Melaleuca quinquenervia	Water	1.96 ± 0.13	13.7 ± 10.1	10.8 ± 4.24	132 ± 169	0.03 ± 0.01	0.06 ± 0.02
	PPV leachate	1.51 ± 0.58	10.8 ± 3.59	9.26 ± 5.00	59.3 ± 21.6	0.02 ± 0.01	0.12 ± 0.08
	WENT leachate	1.35 ± 0.18	7.54 ± 4.08	6.74 ± 0.69	35.0 ± 43.3	0.03 ± 0.01	0.05 ± 0.00
Sapium discolor	Water	1.48 ± 0.00	4.41 ± 1.93	8.55 ± 1.03	58.8 ± 30.6	0.03 ± 0.00	0.06 ± 0.01
	PPV leachate	1.01 ± 0.16	2.54 ± 2.56	6.39 ± 2.53	64.0 ± 27.3	0.02 ± 0.01	0.02 ± 0.01
	WENT leachate	1.51 ± 0.69	6.58 ± 1.27	6.96 ± 0.37	53.7 ± 25.7	0.11 ± 0.12	0.03 ± 0.01
Scolopia chinensis	Water	2.70 ± 1.13	7.13 ± 0.67	10.0 ± 2.97	65.2 ± 2.40	0.02 ± 0.02	0.09 ± 0.01
	PPV leachate	1.88 ± 1.06	1.65 ± 0.46	9.03 ± 5.16	37.5 ± 53.1	0.02 ± 0.01	0.09 ± 0.00
	WENT leachate	2.56 ± 0.19	6.86 ± 1.50	12.7 ± 8.50	36.7 ± 51.8	0.03 ± 0.00	0.08 ± 0.01

3.3.2.4 Incorporating the results of germination tests in leachate irrigation practice

This experiment examined the feasibility of extrapolating phytotoxicity data to estimate the risk of leachate irrigation. It was hypothesized that if tree seedlings and germinating seeds were equally sensitive to landfill leachate, irrigating seedlings with leachate at the EC50 levels would result in 50% of growth inhibition (or deterioration in other health indices). The tree seedlings seem to be less vulnerable to the leachate applied as tree mortality or growth inhibition was not observed in the irrigation experiment. Deviation between the results of germination tests and the irrigation experiment on tree seedlings could be attributed to species differences, sensitivity variation between growth stages and the difference in the method of exposure.

The trees selected for the irrigation experiment are pioneer species which have been commonly used in local enrichment planting (Lau and Fung, 1999). They have a good record of successful plantation in the local climate as they are tolerant to hostile soil conditions and have higher growth rate in response to nutrient addition (Corlett, 1999). Despite species differences, plants are susceptible to salinity during emergence and are become more tolerant as they grow older (Mass, 1993). Germinating seeds in the phytotoxicity test might be more sensitive than the 1 – 2 year old tree seedlings. Furthermore, the volume of leachate supplied was in large excess of the water holding capacity of soil. The surplus moisture was allowed to drain freely from the bottom of the pots and therefore reduced the exposure time to leachate. Soil leaching also prevented the accumulation of salts to phytotoxic levels.

Tree seedlings receiving leachate application at the EC50 levels (determined by the germination tests) did not show any sign of detrimental effects. The plants can be protected from growth inhibition when the leachate irrigation plan is designed in light of phytotoxicity data. It should be noted that EC50 level is only a conservative upper limit of leachate concentration to be applied. It does not imply that EC50 is the optimum application rate or the plants would be severely affected if the application rate exceeds this level. The nutrient requirement of plants in different seasons should also be considered in the leachate irrigation plan. Care should be taken to prevent excessive application of N, which can lead to problems of excessive vegetative growth and pollution.

3.3.3 Soil

3.3.3.1 Initial properties

The loamy sand provided excellent drainage and aeration (Table 3.8). As the weathered granitic materials have not been subjected to plant growth previously, the soil organic carbon was very low (below the detection limit of 1% w/w). The lack of organic matter and clay did not allow nutrients to be retained. The soil material was acidic as bases such as Ca and Mg were released upon weathering. The inherent nutrient reserve was also limited and the supply of macronutrient was inadequate. For horticultural applications, its overall quality could be improved by addition of fertilizer or soil ameliorants such as compost and sewage sludge.

Table 3.8 Properties of soil used for the leachate irrigation experiment.

Parameter	Mean ± SD
pH	5.18 ± 0.31
Electrical conductivity ($\mu S\ cm^{-1}$)	76.8 ± 19.4
Extractable NHx-N ($mg\ kg^{-1}$)	12.4 ± 1.26
Extractable NOx-N ($mg\ kg^{-1}$)	53.9 ± 5.15
Extractable Cl^- ($mg\ kg^{-1}$)	11.6 ± 2.22
Total N ($mg\ kg^{-1}$)	117 ± 3.86
Total P ($mg\ kg^{-1}$)	< 5.00
Organic C (%)	< 0.20
Texture	
Sand (%)	79.1 ± 7.41
Silt (%)	6.91 ± 1.44
Clay (%)	9.59 ± 1.14
Texture class	Loamy sand
Total metals($mg\ kg^{-1}$)	
Na	150 ± 23.4
K	223 ± 82.0
Mg	193 ± 69.6
Ca	433 ± 53.9
Cd	4.98 ± 0.09
Cr	4.21 ± 2.00
Cu	6.29 ± 1.30
Fe	115 ± 24.8
Pb	98.4 ± 15.3
Zn	31.2 ± 10.6

3.3.3.2 Soil reaction (pH)

Perhaps the most outstanding characteristic of the soil solution is its reaction, that is, the soil pH. Microorganisms and higher plants respond markedly to soil reaction because it significantly influences the availability of most of the elements of importance to plants and soil microbes. Low pH has been associated with the release of soluble Al, which can inhibit root development (Marschner, 1991). Most of the landscape plants exhibit stress symptoms for pH below 3.0 (Rorison, 1973) as free acid is present. Low soil pH may also result in nutrient deficiency. Bases such as Ca, Mg and K become more susceptible to leaching loss (Dobermann *et al.*, 1995). P becomes unavailable to plant uptake as it complexes with Al, Fe and their compounds. The direct and secondary effects of acidic soil can result in a unflavorable soil-chemical regime on plants.

The pH of soil before and after leachate irrigation is shown in Table 3.9. The soil used for this study was moderate to strongly acidic. Theoretically, NHx in alkaline leachate would result in an increase in soil pH. However, there was no change in the soil pH of all treatment groups.

Chan *et al.* (1978) reported that the pH of the soil percolate rapidly rose from 4.88 to 9.10 after infiltration with 4 bed volumes of landfill leachate. NHx and other base forming cations such as Ca^{2+} and Mg^{2+} in leachate led to the increase in the pH of soil and soil percolate. Compared with the lime-treated leachate used in the study of Chan *et al.* (1978), the pH of leachates used in the present study was much lower (only 7.52 for PPV leachate and 7.96 for WENT leachate).

Table 3.9 pH, electrical conductivity (EC) and chloride content in soil after 90-day irrigation with leachate from the PPV and WENT Landfills.

	pH	EC ($\mu S\ cm^{-1}$)	Cl^- ($mg\ kg^{-1}$)
Pre-irrigation	5.18 ± 0.31	76.8 ± 19.4	11.6 ± 2.22
Post-irrigation			
Water	5.98 ± 0.91 A	94.7 ± 5.55 c	27.4 ± 11.0 c*
PPV leachate	5.39 ± 0.37 A	823 ± 48.0 b*	68.5 ± 3.47 b*
WENT leachate	5.14 ± 0.41 A	554 ± 113 a*	167 ± 8.39 a*

Means followed with the same letters are not significantly different at $P > 0.05$ by Tukey's test.

* Significant difference ($P < 0.05$) when compared with the initial value using Student's T-test.

The effect of alkaline leachate on soil pH could be counteracted by the exchangeable and the residual acidity of the soil, as well as the H^+ ions produced in microbial nitrification. The enzymatic oxidation is presented very simply as follows (Brady, 1990).

Nitrosomonas:

$$\underset{\text{Ammonium}}{2NH_4^+} \xrightarrow{[O]} \underset{\text{Hydroxylamine}}{2NONH_2} \xrightarrow{-2H^+} \underset{\text{Hyponitrite}}{HONNOH} \xrightarrow{[O]} \underset{\text{Nitrite}}{2NO_2^-} + 2H^+ + \text{energy}$$

Nitrobacter:

$$NO_2^- \xrightarrow{[O]} NO_3^- + \text{energy}$$

Theoretically, 4 moles of hydrogen are produced for each mole of NHx-N oxidized by *Nitrosomonas*. Nitrification of NH_4^+ to NO_3^- can occur within a few weeks in agricultural soils. In field conditions, the net acidity contributed by fertilizer was about 0.08 kg H^+ for each kg of NHx-N applied (equivalent to 1.12 mol H^+ per mol of NHx-N) (Gasser, 1973). Deviation from the stoichiometry of the empirical reaction may be attributed to loss of NHx-N by leaching, volatilization and plant uptake.

Plant uptake of NO_3^- releases bicarbonate ions (HCO_3^-) to maintain the electrical neutrality at the root surface and at the same time counteracts the acidity produced in nitrification. However, the NO_3^- is very susceptible to leaching loss. Leaching, rather than root uptake of NO_3^-, primarily determines the degree of acidification. It is anticipated that if leachate irrigation is continued, nitrification, together with the leaching of NOx-N and base-forming cations, would further lower the soil pH.

Lime application can help regulate the soil pH by neutralizing the acidity produced in nitrification. Also Ca^{2+}which came from the dissociation of lime can help mitigate the imbalanced SAR of leachate and therefore prevent the soil from structural deterioration.

3.3.3.3 Nitrogen

Of the various essential elements, N undoubtedly is the most studied one. N is usually a limiting element in unfertilized ecosystems. The amount of available N in soil is small, while the quantity withdrawn annually by plants is comparatively large. The soil used in this study was deficient in N (Landon, 1991). The total N content was only 117 mg kg^{-1} (Table 3.10), of which only 12.4 mg kg^{-1} of NHx-N and 53.9 mg kg^{-1} of NOx-N was available for plant uptake.

Since *Acacia auriculiformis* and *Casuarina equisetifolia* are N-fixing plants, soil planted with them was sampled and analyzed separately for those planted with non-N-fixing species. In the water treatments (controls), their seedlings led to significant elevation in the levels of soil TN, while significant increase in NHx-N content was only observed in soil planted with *Casuarina equisetifolia* (Table 3.10).

Compared with N-fixation, leachate application had a much larger influence on the levels of soil nitrogen. There were marked increases in soil N content in leachate treatments (Table 3.10), suggesting that substantial amount of N in the leachate may be retained in the soil. The levels of TN and NHx-N increased by 4 times after treatment with diluted WENT leachate. When compared within species, the soil TN content in WENT leachate treatments was 2 to 3 times that of the control. Within

Table 3.10 Amount of total and extractable N contents in soil before and after 90-day irrigation with water and leachate from PPV and WENT Landfills.

	Treatment	NHx-N ($mg\ kg^{-1}$)	NOx-N ($mg\ kg^{-1}$)	Total N ($mg\ kg^{-1}$)
Pre-irrigation		12.4 ± 1.26	53.9 ± 5.15	117 ± 3.86
Post-irrigation				
Casuarina equisetifolia	Water	15.1 ± 1.57 b *	48.9 ± 11.5 b	179 ± 11.5 b *
	PPV	17.6 ± 2.22 b *	180 ± 41.3 b *	224 ± 28.8 b *
	WENT	46.7 ± 28.5 a *	496 ± 190 a *	501 ± 65.6 a *
Acacia auriculiformis	Water	14.3 ± 1.39 a	38.2 ± 11.7 c	271 ± 74.7 b *
	PPV	13.5 ± 1.11 a	140 ± 18.6 b	270 ± 37.2 b *
	WENT	19.1 ± 4.68 a *	399 ± 49.5 a *	505 ± 76.1 a *
Non N-fixers	Water	14.9 ± 3.93 b	34.9 ± 11.0 b	157 ± 33.9 c
	PPV	14.6 ± 1.53 b	168 ± 23.1 b *	267 ± 62.2 b *
	WENT	60.0 ± 15.2 a *	429 ± 196 a *	531 ± 69.7 a *

When compared within species, means followed by the same letters are not significantly different at $P > 0.05$ by Tukey's test.

* Significant difference ($P < 0.05$) when compared with the initial value using Student's T-test.

leachate treatments, significant differences between N-fixers and non-N-fixing trees were not observed. The effect of N-fixation may be masked by the ample supply of N from leachates. Moreover, the N-fixation activity may be inhibited by the excessive N or other constituents in leachate.

Over a period of 90 days, application of PPV and WENT leachates at their respective EC50 provided 473 and 331 kg N ha^{-1} of readily available NHx-N to the soil. This input can fulfill the annual N requirement of a tropical ecosystem (400 kg N ha^{-1} y^{-1}) (Bradshaw, 1983). If leachate is applied all year round, the dosage can be further reduced. The risks associated with leachate phytotoxicity can be further reduced without compromising the plant nutritional needs.

The reducing condition in the landfill body is unfavorable to nitrification, resulting in low NOx-N in the two leachate samples (<1 mg L^{-1}). However, the soil NOx-N content increased remarkably after irrigation for 90 days. When leachates were applied to the soil, the good aeration in the sandy loam soil provided a favorable condition for nitrification to proceed. A portion of NH_4^+ in leachate was transformed to NO_3^-.

In energy terms, assimilation of NH_4^+ is more efficient than NO_3^-, since NO_3^- requires reduction to NH_4^+ before incorporation into the synthesis of amino acids. However, NHx is toxic to aquatic life. It is also phytotoxic when applied in excess. Nitrification helps reduce the harmful effects of the leachate applied, as NO_3^- is much less toxic to aquatic life when compared with NHx.

Transformation of NH_4^+ to NO_3^-, however, may lead to another pollution problem. Unlike NH_4^+, anionic NO_3^- has a very low retention in soil (Brady, 1990; Rowell, 1996; Snyder, 1996). It can be leached with the soil percolate and contaminate water bodies nearby. High levels of NO_3^- can cause eutrophication in open waters. NO_3^- pollution in groundwater can be even harmful to human as intake of NO_3^- in drinking water can lead to methemoglobinemia (Stevenson and Cole, 1999).

The impact of NO_3^- pollution is less serious in modern sanitary landfills since most of them are equipped with drainage and treatment systems to handle surplus moisture in the final soil cover. However, it has become a concern on some old landfills with limited control measures on run-off and soil percolate; or when the site of leachate application is not within the landfill area, for example, when landfill leachate is applied to nearby borrow areas during restoration.

Besides proper run-off collection, the risk of NO_3^- pollution can be mitigated by determining the leachate application rate carefully to prevent excessive supply of N. Different soil and plant analytical methods have been suggested for determining the actual quantity of fertilizer necessary under specific field conditions (Bell, 1999). Soil in the field is sampled for chemical analysis. Pot trials which investigates the response of plants to different application rates of soil amendments can give information on the amount of N input required for optimal plant growth. Soil testing provides information on fertilizer requirements prior to planting, which ensures the rapid establishment of vegetation.

Plant tissue analysis, giving the N concentration in specific plant parts, can be used for fertilizer application decision-making. It helps determine the nutrient status of plants and, if necessary, indicates the timing and quantity of fertilizer application. Recent innovation in plant analysis techniques have greatly reduced the amount of plant tissue to be harvested. Moreover, non-destructive methods such as leaf reflectance have been developed to reflect the nutrient status of plants (e.g. Waskom *et al.,* 1997).

Appropriate application of N should be based on optimal economic returns and minimum damage to the environment. The available N derived from leachate replaces equivalent amounts of fertilizers to be applied.

3.3.3.4 Phosphorus

P is another essential element influencing plant growth and production. Unlike N, this element is not supplied by biochemical fixation, but must come from other sources (such as mineralization of litter and fertilizer) to meet plant requirements. The P contents of natural soil materials are usually less than 1 mg kg^{-1} (Jim, 1996).

The native P compounds are mostly highly insoluble, which are unavailable for plant uptake. Furthermore, when available forms of P are added, either by fertilizer application or waste effluent disposal, they are changed to unavailable complexes and become highly insoluble. It is generally known that when sewage effluent is disposed of by irrigation, the P in the effluent will be retained by soil through adsorption or precipitation after the formation of water insoluble compounds (Menzies *et al.*, 1999).

The levels of total P in the soil were below the detection limit (< 5 mg P kg^{-1}) before and after leachate application. Compared with domestic sewage, leachate only provided a limited amount of P to soil-plant system. Although a major portion of P was applied in the form of readily available orthophosphate, it would be quickly depleted by plant uptake (in rapid vegetative growth in response to N supply), leaching and complexing with Al, Fe and their compounds in acid soils.

The fixation of P through reactions in acid soil can be minimized by holding the soil pH between 6 and 7. However, P is still deficient in the long run. A tropical ecosystem requires a supply of 36 kg P ha^{-1} y^{-1} (Bradshaw, 1983). Supplement with inorganic P or P-rich soil amendments such as sewage sludge may be beneficial, before P becomes a limiting factor for plant growth. Landfill leachate supplemented with inorganic P significantly improved the growth of plants in leachate irrigation (Fu, 2004).

3.3.3.5 Conductivity

Increases in soil salinity and sodicity are of special interest in arid and semi-arid environments with high evapotranspiration, where excessive salts in soil are not easily leached out. Only a few studies in the literature addressed the effects of leachate irrigation on soil salinity. The electrical conductivity of landfill leachate, which ranged 3 - 20 mS cm^{-1} (samples collected in November 2001) (see Table 3.3), was much higher than that of high salinity water (0.75 - 2.25 mS cm^{-1}) (Landon, 1991). Landfill leachate contains considerable amounts of dissolved salts and it has been demonstrated that irrigation with landfill leachate resulted in soil salination (Wong and

Leung, 1989; Hernández *et al.*, 1999). In the present study, although the soil did not become saline in all treatments, the significant increase in electrical conductivity of the soil extract (EC) after leachate application was dramatic (Table 3.9).

Soil salination as a consequence of leachate disposal also implies exotoxicological effects on plants (McBride *et al.*, 1989; Pastor *et al.*, 1993) and soil organisms (Garcia and Hernández, 1996). Soil salination has negative effects on microbial respiration, especially when Na^+ and/or Cl^- are involved. Microbial activity and the cycling of nutrients may be inhibited. Furthermore, high soil salinity in newly restored sites may favor salt tolerant species and may alter the succession of the ecosystem.

A graphic representation of the effects of salinity on crop yield is shown in Figure 3.4. Soil is considered to be saline if the EC of its water extract exceeds 4 mS cm^{-1} (Landon, 1991). Sensitive crops begin to be affected above this level of salinity. They can be harmed by osmotic stress as well as the toxic effects of specific ions such as Na^+, NH_4^+ and Cl^-. Increased salinity in this study did not result in any observable detrimental effects. However, care should be taken to control the amount of salts in soil pore water, especially in field application of leachate. Salts may accumulate and eventually lead to vegetative damages unless they are leached away. The potential hazard may be exacerbated by drought, when salts accumulate in soil.

Besides minimizing the salt input by a proper dilution rate of leachate, the impact of salts can be reduced if salts were eluted away from the rooting zone. Ayers and Westcot (1985) proposed the concept of a leaching requirement. It is the excess

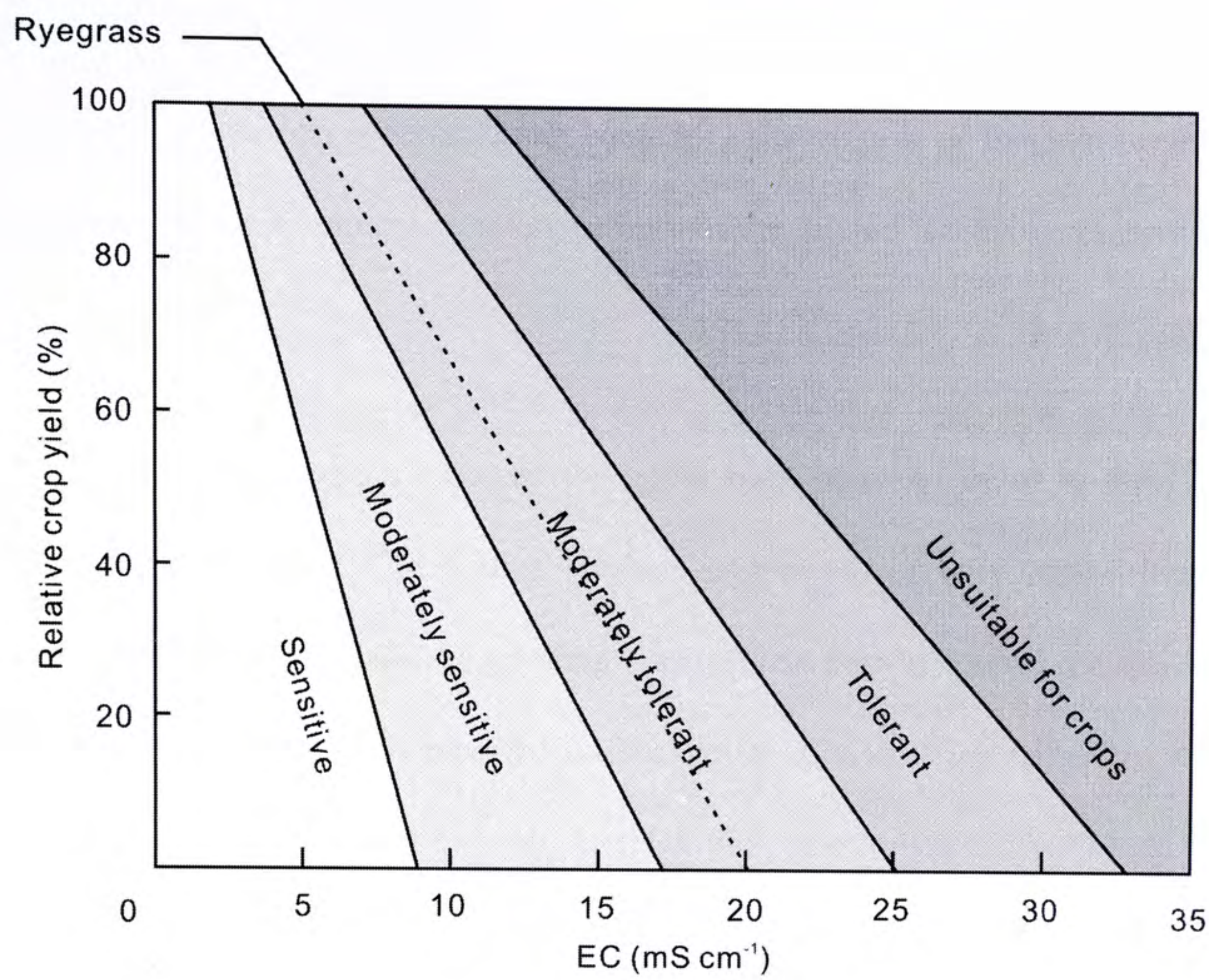


Figure 3.4 Relationship between the relative crop yield and electrical conductivity of soil extracts. Taking ryegrass (which is denoted by segmented line) as an example, this moderately tolerant species has maximum (100%) yield when soil salinity is below 7 mS cm^{-1}. Yield decreases with increasing soil EC and ryegrass cannot grow in soil with salinity greater than 20 mS cm^{-1} (adapted from Mass, 1986).

amount of water, in addition to evapotranspiration, required to leach salts out of the rooting zone to maintain an acceptable salt content. The volume of irrigation can be calculated based on the evapotranspiration, salinity of the diluted leachate and the crop tolerance.

If the leaching requirement exceeds the upper limit of water input to the final cover, alternating irrigation with diluted leachate and water, or only apply diluted leachate in rainy seasons (usually the growing seasons) can help to control the soil salt content. Bowman *et al.* (2002) reported the feasibility of alternating irrigation with leachate and water on grasses (*Cynodon dactylon* and *Pennisetum clandestinum*) to mitigate the problem of soil salination. Leachate applied at 50% (one leachate irrigation followed by one watering) resulted in yield reduction, but the yield was better than the control (water only) treatment when the frequency of leachate application was reduced to 20%. The irrigation practice employed by Bowman *et al.* (2002) can be further improved with the consideration of the phytotoxicity of leachate, the N requirement and the leaching requirement of soil to avoid excessive application of N and salts.

Compared with applying leachate continuously, alternating irrigation may be a better option. Keeping the N input rate unchanged, the two irrigation plans would add the same amount of salt to soil. However, the alternating irrigation plan can leach away the salts more efficiently. In other words, more salts can be leached out using smaller volumes of water. Thus, the hydraulic loading to the final soil cover can be reduced. It should be noted that although the application of excess water can mitigate salination problems, plants would be severely impaired when the soil is water-logging with high salinity water, especially with leachate (Shrive and McBride, 1995). Excessive water should be drained out of rooting zone. Moreover, planting salt-tolerant species (e.g. *Hisbicus tiliaceus* which was tested in the present study) can lower the adverse impact to vegetation in episodic drought conditions due to hot dry weather or a breakdown of the irrigation system.

3.3.3.6 Chloride

The change in the soil Cl^- content has been seldom addressed in research on sewage irrigation. However, it becomes a concern in leachate irrigation because Cl^- is one of the prevalent anions in landfill leachate. The levels of Cl^- in soil were significantly increased after leachate application (Table 3.9). The soil Cl^- after WENT leachate treatment was nearly ten times that of the initial level.

Besides osmotic stress, Cl^- can exhibit specific ion toxicity to plants. Levels up to 10 meq L^{-1} (equivalent to 350 mg L^{-1}) in the soil extract would be detrimental to woody plants. The effects may be worse with the use of sprinklers. Cl^- concentration in sprinkling water of 3 meq L^{-1} (equivalent to 105 mg L^{-1}) caused leaf burnt (Landon, 1991). Each 10 mM increase in Cl^- in irrigation water reduced the foliage biomass of *Salix viminalis* by 3% (Stephens *et al.*, 2000).

The levels of soil Cl^- in the two leachate treatments exceeded the threshold level described in Stephens *et al.* (2000). However, no growth inhibition was observed in the present study. Leachate treatment added Cl^- as well as N to soil. Growth promotion by nutrient supply might outweigh the inhibitory effect of Cl^-. However, it should be noted that the potential hazard of Cl^- ions, as well as osmotic stress, may be exacerbated when salts are left behind by evapotranspiration.

As mentioned, Cl^- may be one of the major plant toxicants because of it high concentration in landfill leachate. Toxicity reduction by removing Cl^- from wastewater is difficult as it cannot be removed from wastewater by conventional physical and biological treatment technology. Cl^- has to be removed by reverse osmosis. However, a large scale operation is not economically practicable.

The only way to control the detrimental effects of Cl^- is diluting it to a concentration causing no adverse effects (the NOAEL, on the recipient plants or even lower). Also, it is necessary to prevent it from accumulating in soil and being concentrated in drought. Irrigation should be applied in excess of evapotranspiration to provide sufficient leaching.

3.3.3.7 Metals

Table 3.11 presents the total metal contents in soil before and after leachate treatments. Due to their potential phytotoxicity and food-chain effects, certain heavy metals (e.g. Cd, Pb and Zn) are of special interest. An increase in soil metal contents had been reported in some studies with leachate irrigation (Winant *et al.*, 1981; Chan, 1982; LaBauve, 1988; Hernández *et al.*, 1999). Since the leachates from local landfills contained only trace amount of heavy metals, it is no wonder that there was no significant change in the soil contents of heavy metals after leachate irrigation.

Table 3.11 Soil metal content before and after 90-day irrigation with water and leachate from PPV and WENT Landfills.

Metals	Total content ($mg\ kg^{-1}$)			
	Pre-irrigation	Post irrigation		
		Water	PPV leachate	WENT leachate
Na	150 ± 23.4	184 ± 42.7	208 ± 20.1	179 ± 46.9
K	2230 ± 820	3200 ± 332	3410 ± 571	3520 ± 751
Ca	433 ± 53.9	349 ± 50.0	367 ± 96.9	366 ± 84.5
Mg	193 ± 69.6	287 ± 69.3	271 ± 52.8	257 ± 42.4
Cd	4.98 ± 0.09	1.43 ± 2.25	0.63 ± 0.18	1.96 ± 2.88
Cr	4.21 ± 2.00	2.63 ± 0.84	5.08 ± 2.14	9.41 ± 9.68
Cu	6.29 ± 1.30	6.60 ± 1.14	8.19 ± 1.24	8.38 ± 1.90
Fe	1150 ± 248	1310 ± 61.4	1310 ± 90.8	1330 ± 38.9
Pb	98.4 ± 15.3	101 ± 20.0	110 ± 13.5	106 ± 20.4
Zn	31.2 ± 10.6	38.5 ± 5.54	45.8 ± 7.68	37.7 ± 8.59

However, the levels of Na and K, which were present at high levels (1960 mg Na L^{-1} and 1330 mg K L^{-1} in WENT leachate) in the leachates, were not significantly elevated in the leachate-treated soil. This may be attributed to the low CEC of soil, presence of competitive ions and removal by leaching.

Retention of metals in soil can be accomplished by (but not limited to) cation exchange on clay particles. However, the decomposed granite used in this study had a poor holding capacity for exchangeable cations. The cation exchange capacity (CEC) of natural decomposed granitic soil ranged only from 8.5 - 11.7 cmol kg^{-1} (Jim, 1996). It can be further reduced with soil acidification as a consequence of nitrification. Exchange sites on soil particles might be saturated quickly at the beginning of leachate irrigation and thereby eliminating the capacity for accepting/retaining cations added in the subsequent irrigations.

Moreover, other cations, such as ammonium (NH_4^+) and hydrogen (H^+) ions, could compete with metal ions for the soil exchange sites. The cations were retained in order of $Al_3^+ > Ca^{2+} > Mg^{2+} > K = NH_4^+ > Na^+$ (for major cations) (Chan et al., 1978; Chan, 1982; Brady, 1990) and Pb > Zn > Cd > Ni (some heavy metals) (LaBauve *et al.*, 1988). Although the exchange selectivity of NH_4^+ is not the highest among cations, the concentration of NH_4^+ in leachates was much higher than metals. The ratio of [NHx-N]:[Na] was approximately 1:1 in the WENT leachate. However, the ratio of [NHx-N]:[Fe] in the WENT leachate was up to 2000:1. When leachate was applied to soil, large amounts of NH_4^+ competed with other cations and occupied a considerable portion of the ion exchange sites.

Huge amounts of NH_4^+ were continuously applied with leachate irrigation. Metals with lower selectivity would be eventually replaced/exchanged and dissolved in soil pore water. Since surplus moisture was allowed to drain freely, dissolved metals which could not be retained by cation exchange were eluted. It is anticipated that 'replacement' of base-forming cations such as Ca^{2+} and Mg^{2+} with NH_4^+ not only led to loss of plant nutrients but also exacerbated soil acidification.

3.4 Conclusions

Germination tests provided a conservative estimate of the toxicity level of leachate samples. With due consideration of the phytotoxicity, irrigation plan can be designed to safeguard plants from toxic effects while at the same time meeting the N demand for plant growth.

Most of the leachate-irrigated plants were benefited by the ample supply of NHx-N. Plant species like *Hibiscus tiliaceus* and *Litsea glutinosa* were greatly stimulated in growth by leachate application. The vigorous plant development indicated that the inhibitory substances in soil were kept within the tolerance limits of tree seedlings.

With proper dilution, leachate from sanitary landfills seems to be a good alternative source of N, especially when heavy metal contents were low. The levels of N and probably some macronutrients such as K seem to be sufficient, but the low concentration of P is still a concern. Addition of P fertilizers would be required in a long term perspective to compensate for plant requirements.

However, the risk of salt accumulation requires special attention. It should be noted that the problems associated with soil salination would be exacerbated by drought conditions. To assure the sustainability of leachate irrigation, adequate water should be applied to fulfill the leaching requirement so as to prevent the accumulation of salts, and thus preventing sodicity from rising to unacceptable levels.

Furthermore, there is a concern that leaching of base-forming cations, together with nitrification, results in soil acidification. Lime application not only mitigates the problems associate with acid soil, but also remedies the Na imbalance (high SAR) in the applied leachate. Leaching of excess N, especially NOx-N, may contaminate the water downstream. The rate of application should be carefully calibrated to meet the N demand without compromising the environment.

Chapter 4 Fate and distribution of N after soil application of landfill leachate

4.1 Introduction

4.1.1 The needs of external N supply in ecological restoration

Restoration of derelict lands by natural processes occurs slowly. It can take up to 100 years to achieve a satisfactory vegetative cover. However, when there is an urgent need such as soil stabilization, the natural processes have to be accelerated. A stable vegetative cover may need to be established within a few years. Experience has shown that N is commonly an important limiting factor in land remediation. It is the nutrient required in the greatest amount by living tissues. Adequate amounts of N must therefore be available in soils if plants are to grow properly. However, it does not come from soil minerals like other nutrients. It is accumulated in the soil surface, mostly by biological fixation and the return of biomass N through litter fall.

A satisfactory self-sustaining vegetation cover in temperate regions requires a minimal reserve of 1000 kg N ha^{-1} (Bradshaw, 1983). However, it is not always feasible to retain the original topsoil. On some degraded land, the organic matter, as well as the N stored with it, may be completely lost. No more than 100 - 200 kg N ha^{-1} may be present at the beginning of revegetation. N deficiency may lead to poor growth and recession. Problems connected with degraded lands like soil erosion may reoccur. The required reserve of N has to be rebuilt as rapidly as possible. Even if the top soil is replaced, the original soil conditions cannot be restored. Anaerobic conditions in the stockpiled top soil generates high levels of NHx. Nitrification in the aerobic zone of stockpiled top soil liberates large amount of NO_3^-, which is susceptible to leaching loss. Davies *et al.* (1998) demonstrated that the soil N content fell to below 1% w/w in the first 18 months after replacement of stockpiled soil and leaching accounted for an N loss of 2800 kg N ha^{-1}. Supply of N from artificial sources is usually required.

The previous chapters suggested the use of landfill leachate as an alternative to mineral fertilizers. The irrigation experiment has shown that phytotoxicity tests using germinating seeds provided a safe upper limit of the application rate. However, whether the application rate is optimal for the reconstruction of N capital depends on the ability of the ecosystem to take up and store the applied N in organic matter. Research on the excessive use of manure and mineral fertilizer has reported considerable detrimental effects. An ecosystem could adsorb N deposition of 730 kg N ha^{-1} over a 20-year period without major change in soil N metabolism (Nilsson *et al.*, 1988). However, twice the amount of N induced nitrification, soil acidification and an increased level of aluminum in soil pore water. Moreover, the excess deposition of NHx has been identified as one of the principal causes of forest decline.

4.1.2 Objectives of study

A limited amount of research has focused on nutrient reuse for revegetation purposes since leachate irrigation technology has long been considered a wastewater treatment or disposal. Chapter 3 discussed the responses of plants and soil to the elevated N supply from leachate. This chapter evaluates the efficiency of N usage, when the leachate application rate was determined solely based on toxicological information.

Often an N budget, or a mass balance, approach is needed to understand the options to improve N management. It provides a valuable framework to quantify and examine the N input and loss in a soil-plant system. Two general approaches have been used in N balance studies. One uses a ^{15}N-labelled input, from which a balance is calculated for the labelled ^{15}N (Barraclough, 1995; Stevenson and Cole, 1999). It focuses on the fate of the labeled ^{15}N and can improve tracing sensitivity in fertilizer N balance work (Legg and Meisinger, 1982), but provides little information about the behaviour of the existing N in the system. Another approach involves a completed budget and documents the input and output of all forms of N, including the N incorporated into biomass. In the present study, the latter method was adopted with a soil column design to investigate the accumulation and distribution of the applied N in plant tissues and within soil profiles.

It is hoped that a better understanding on the role and fate of leachate N in the soil-plant system can provide guidelines about the efficient use of leachate for revegetation purposes. Improved efficiency can be achieved by using less N or keeping the applied and residual N within the soil-plant system by limiting transport processes (leaching, runoff, soil erosion and volatilization).

4.2 Materials and methods

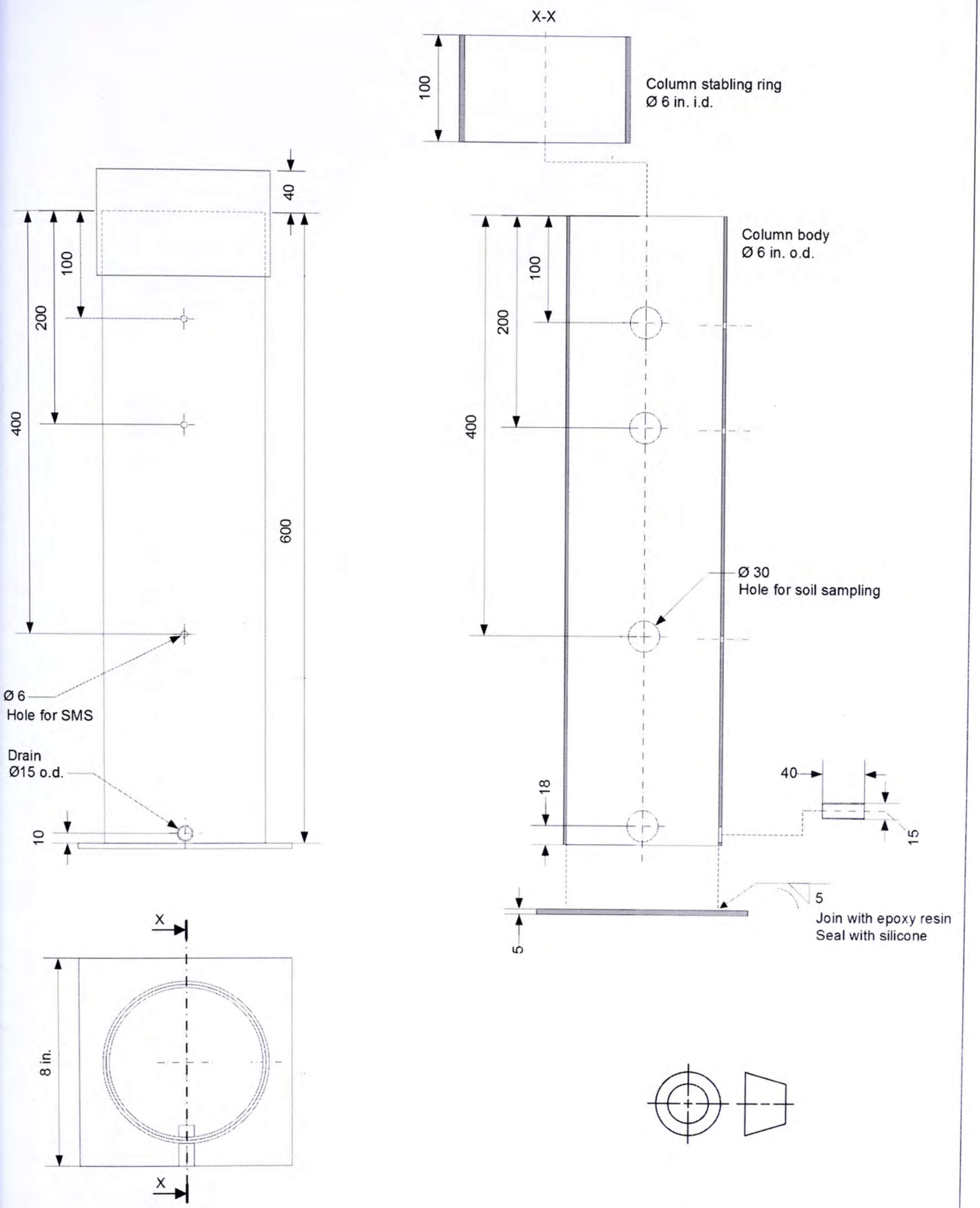
A soil column experiment was conducted under greenhouse condition to investigate the distribution of leachate N. Four species that included commonly used trees (*Hibiscus tiliaceus* and *Litsea glutinosa*) and grasses (*Paspalum notatum* and *Vetiveria zizanioides*) were planted in PVC columns packed with a completely decomposed granite. Columns without vegetation were also included for comparison. Leachate samples from a local landfill were first evaluated for phytotoxicity using seed germination/root elongation tests. Soil columns were then irrigated with leachate diluted to the EC50 level. Plant growth and the composition of column percolate were monitored throughout the period of leachate irrigation. Mineral contents of biomass and soil were measured at harvest. The results were compared with treatments with the application of mineral fertilizer.

4.2.1 Leachate

Leachate from the WENT Landfill was collected in late January, 2003. The samples were stored in air-tight 10-L PVC carboys and stored at 4°C. Chemical analysis and phytotoxicity tests were conducted immediately using methods as described in Chapter 2.

4.2.2 Soil column

The soil columns consisted of PVC cylinders with 15 cm i.d. and 60 cm height attached to a PVC bottom sheet (Figure 4.1; Plate 4.1). A drainage tube was mounted at the bottom of each column to collect soil percolate to a 1-L PE bottle.



Drawing title 圖則名稱		Department of Biology 香港中文大學 生物系 The Chinese University of Hong Kong	
Soil column			
	Drawn by 製圖: M. Cheng	Date 日期: 03 Aug 2004	
Project/Tender 項目/標書	Checked by 審核	Scale 比例: N.T.S.	
Utilization of landfill leachate as an alternative source of plant nurients	CAD Ref. 電腦記錄: \..\phase3\soil_column.vsd		

Plate 4.1 Soil columns arranged in a randomized block layout in a greenhouse. The photo was taken prior to harvesting.

Figure 4.1 (on page 125) Drawing showing the design of soil columns. All dimensions in mm unless otherwise specified.

4.2.3 Plant selection and establishment

Tree species (*Hibiscus tiliaceus* and *Litsea glutinosa)* were chosen for this study because of their good growth performance under leachate application in the previous experiment (Chapter 3). In addition, two grass species (*Vetiveria zizanioides* and *Paspalum notatum* were chosen to study the N budget of the soil-plant systems with different vegetative cover.

The value of *Vetiveria zizanioides* is gaining recently, especially for soil stabilization. It is one of the 'coarse perennial grasses' of the tribe Andropogoneae (World Bank, 1993). There are at least two known species of vetiver, *Vetiveria zizanioides* and *Vetiveria nigratana*. The planting of *Vetiveria zizanioides* is more common, owing to its lower potential of becoming a weed. It is a dense, clumping perennial grass of 1.5 m tall. It grows well in a broad range of soil pH (4 to 11) and soil with relatively high salinity (20 mS cm^{-1} with 50% yield reduction) (Troung, 1994). Field studies have shown that *Vetiveria zizanioides* can grow well in the local climate (Hill and Peart, 1999).

The World Bank is actively promoting *Vetiveria zizanioides* as a soil and water conservation tool. The dense and deep root system significantly reduces soil and nutrient loss and improves soil moisture and ground water (World Bank, 1993). In India, *Vetiveria zizanioides* is widely used in the sugar cane fields as contour conservation hedges and for the stabilization of slopes, roadsides and embankments.

Recent research explored the uses of *Vetiveria zizanioides* in wastewater treatment and phytoremediation. Nutrient uptake by fast growing *Vetiveria zizanioides* can greatly reduce N and P from eutrophic pond water (Zheng *et al.*, 1997) and landfill leachate (Xia *et al.*, 1999, 2000; Percy and Truong, 2003). Moreover, the high transpiration rate can reduce the volume of wastewater to be discharged. In this study, *Vetiveria zizanioides* was tested against leachate irrigation in a soil column experiment to evaluate its efficiency in controlling the leaching of nutrients and to compare it with another grass species (*Paspalum notatum)* which is commonly used in hydroseeding mixes.

Treatment groups without plants were compared with those with vegetation. Columns without vegetation were packed with soil to a 60-cm depth. The soil was a decomposed granite (DG) collected from the borrow area of Lam Tei Quarry, Tuen Mun, which was passed through a 5-mm mesh sieve to remove large particles. Different methods, which are similar to those used in enrichment planting, were adopted to establish the plants in soil columns.

Seedlings of *Hibiscus tiliaceus* and *Litsea glutinosa* of about 30 cm height (about 1 year-old) were purchased from government tree nurseries. Bulk soil was gently removed and the tree seedlings were transplanted into the columns. Pa*spalum notatum* was established by germination inthe soil columns. The columns were filled to the top (60 cm depth). Seeds (0.2 g, equivalent to 11 g m^{-2}) were sown on the soil surface and covered with a thin layer of soil. Prior to seed germination, the top soil surface was covered with aluminum foil to conserve soil moisture. After germination, Nitrophoska (N:P:K 15:15:15) was applied at the rate of 20 kg N ha^{-1} to facilitate early seedling growth. *Vetiveria zizanioides*, which does not produce seeds that germinate under normal field conditions (Hopkinson, 2002), was established in the soil columns in accordance with the method of the National Research Council (1993). Clumps of *Vetiveria zizanioides* were obtained from a nursery and were divided into slips with about 5 tillers. Before transplanting, the leaf tops were cut off to 20 cm and the root base was trimmed to 10 cm to reduce transpiration during the initial growth.

After establishment, all plants acclimated in greenhouse for not less than 1 month. Each column, including the columns without vegetation, received water irrigation at 12 mm three times per week. Soil percolate was allowed to drain freely. Three columns of each species were selected randomly one day before the first leachate irrigation for the determination of initial biomass and N content.

4.2.3 Leachate application

The soil columns then received applications of artificial fertilizer or diluted leachate. The N-P-K fertilizer Nitrophoska (15:15:15) was distributed on the soil surface at a rate of 200 kg N ha^{-1} after acclimation. Columns destined for leachate treatment received leachate irrigation at the EC50 level determined by the phytotoxicity tests. Treatment group without leachate and fertilizer were included for comparison. Each column in the leachate treatment received 12 mm of diluted leachate 3 times a week while the same amount of tap water was applied to the columns of the fertilizer treatment and the control. Each treatment had four replicates arranged in randomized blocks in a greenhouse. Pesticide and fungicide were applied only when necessary.

Irrigation lasted for 12 weeks. Soil percolate collected was stored at 4°C, after volume determination by the gravimetric method. Percolates collected each week were pooled for chemical analysis, using the methods described in Chapter 2.

4.2.4 Post irrigation harvesting and analysis

After harvesting the aboveground biomass, the columns were perforated at levels of 10, 20, 40 and 60 cm measured from the top of column to collect the soil samples at different depths. Soil oxidation-reduction potential (ORP) was measured *in-situ* by inserting a combined ORP electrode (Orion 9678BN, Orion Research Inc., Boston, USA) into the moistened soil. Soil samples were air-dried and analyzed chemically, following the methods described in Chapter 3. The underground biomass in the columns were harvested after the collection of soil samples.

4.3 Results and discussion

4.3.1 Leachate

The high strength leachate from the WENT Landfill was chosen for this experiment. Its strength was higher than the samples collected for the previous experiments (Table 4.1), possibly because of the lower rainfall in dry seasons. It was characterized by high levels of COD and NHx, while the concentrations of heavy metals were relatively low compared to those reported in the literature (e.g. Tchobanoglous *et al.*, 1993). With a NHx-N content of 5070 mg N L^{-1}, each m^3 of raw leachate used in this study could provide 5 kg of N, which was readily available for plant uptake.

Table 4.1 Properties of leachate sample used in the soil column experiment.

Parameters	Mean ± SD
pH	8.23
Electrical conductivity	57.8
COD	7500 ± 90.9
TOC	1770 ± 18.5
Total Kjeldahl nitrogen (TKN)	5990 ± 62.9
Ammoniacal nitrogen (NHx-N)	5070 ± 291
Oxidized nitrogen (NOx-N)	< 1.00
Total phosphorus (TP)	22.6 ± 1.89
*ortho*phosphate phosphorus (PO_4^{3-}-P)	15.4 ± 0.21
Chloride (Cl^-)	5460 ± 61.5
Total metals	
Sodium (Na)	2550 ± 31.0
Potassium (K)	1740 ± 20.0
Calcium (Ca)	19.7 ± 0.17
Magnesium (Mg)	15.7 ± 2.21
Cadmium (Cd)	< 0.001
Chromium (Cr)	< 0.001
Copper (Cu)	< 0.001
Iron (Fe)	4.56 ± 1.89
Manganese (Mn)	< 0.001
Lead (Pb)	< 0.001
Zinc (Zn)	1.85 ± 0.29

All units in mg L^{-1} except for pH (no unit) and electrical conductivity (mS cm^{-1}).

4.3.2 Plants

4.3.2.1 Growth

Figure 4.2 presents the percentage change in biomass after treatment with leachate and fertilizer. Compared with the control group with water irrigation only, the trees exhibited better growth both in the leachate and fertilizer treatments. Trees receiving leachate and fertilizer at least tripled their aboveground biomass in 12 weeks. They were taller, with larger leaf number of larger foliage area (Plates 4.2 and 4.3). The findings were consistent with the previous experiment (Chapter 3).

Although the amount of N applied by leachate (1920 kg N ha^{-1}) was nearly 10 times that in the fertilizer treatment, the biomass gains in leachate treatments were not significantly greater. This may be attributed to nutrient saturation and the effects of inhibitory factors. The N application rate of 200 kg N ha^{-1} in the fertilizer treatment can theoretically support the annual growth of a temperate ecosystem (Bradshaw, 1983). The amount of available N in the soil column was no longer limiting in a given weather condition and water supply. The growth did not increase by increased supply. Moreover, some inhibitory constituents in leachate, such as excessive amount of NHx, Cl^- and Na^+, might weigh out a part of the beneficial effects of leachate. The growth performance of trees in the leachate treatment suggested that, although the EC50 of germination tests provided a safe upper limit of the application rate, a lower application rate should be considered when plant nutrient requirements are fulfilled.

The aboveground and underground biomass gains of *Paspalum notatum* were 1213% and 1720%, respectively, which were the highest among the 4 species.

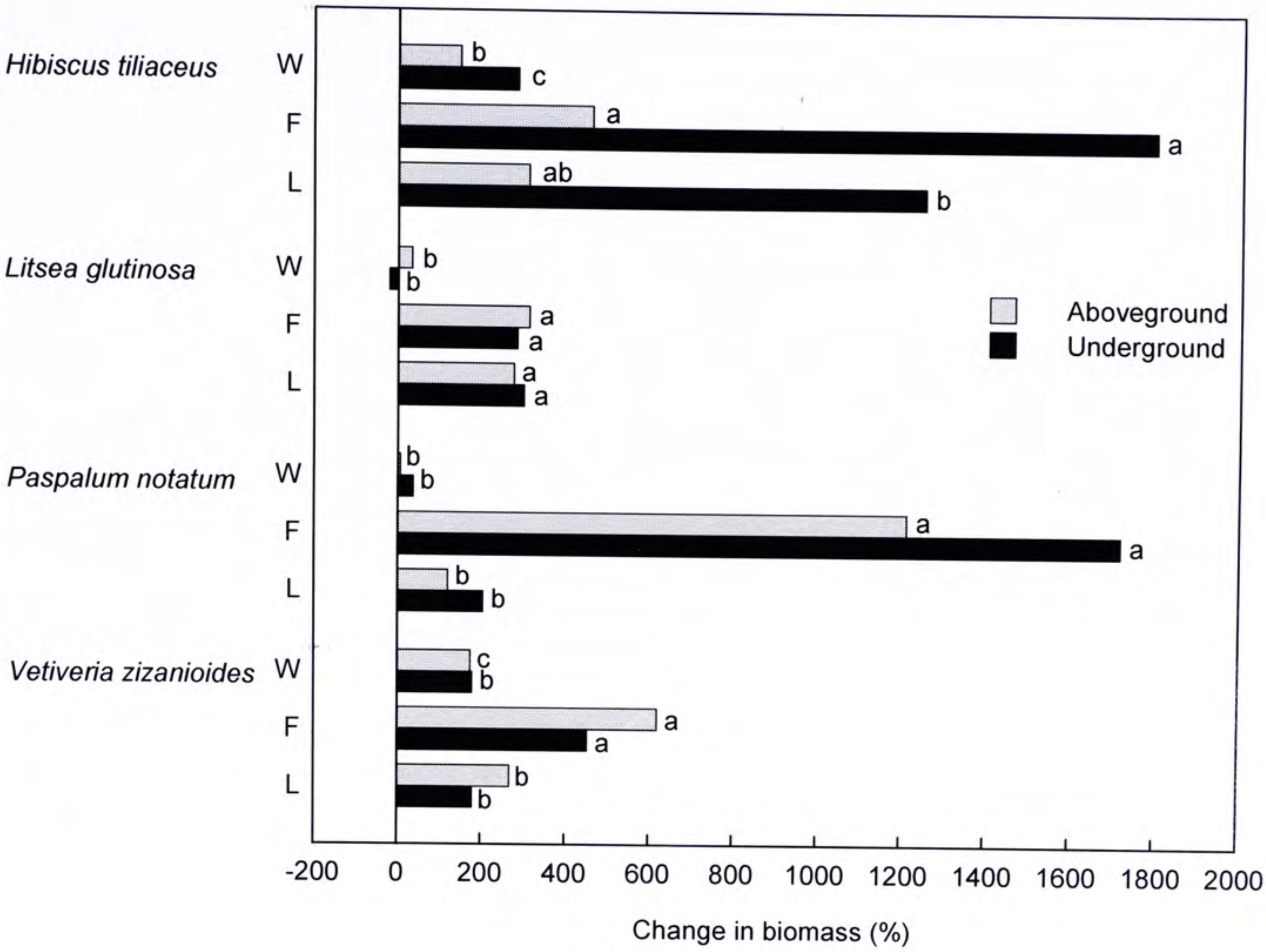


Figure 4.2 Plant growth in biomass after 12-week treatment with water only (W), fertilizer with water irrigation (F) and leachate (L). When compared within species, bars followed by the same letters are not significantly different at P > 0.05 by Tukey's test.

Plate 4.2 *Hibiscus tiliaceus* after receiving water irrigation, fertilizer with water irrigation and leachate application for 12 weeks.

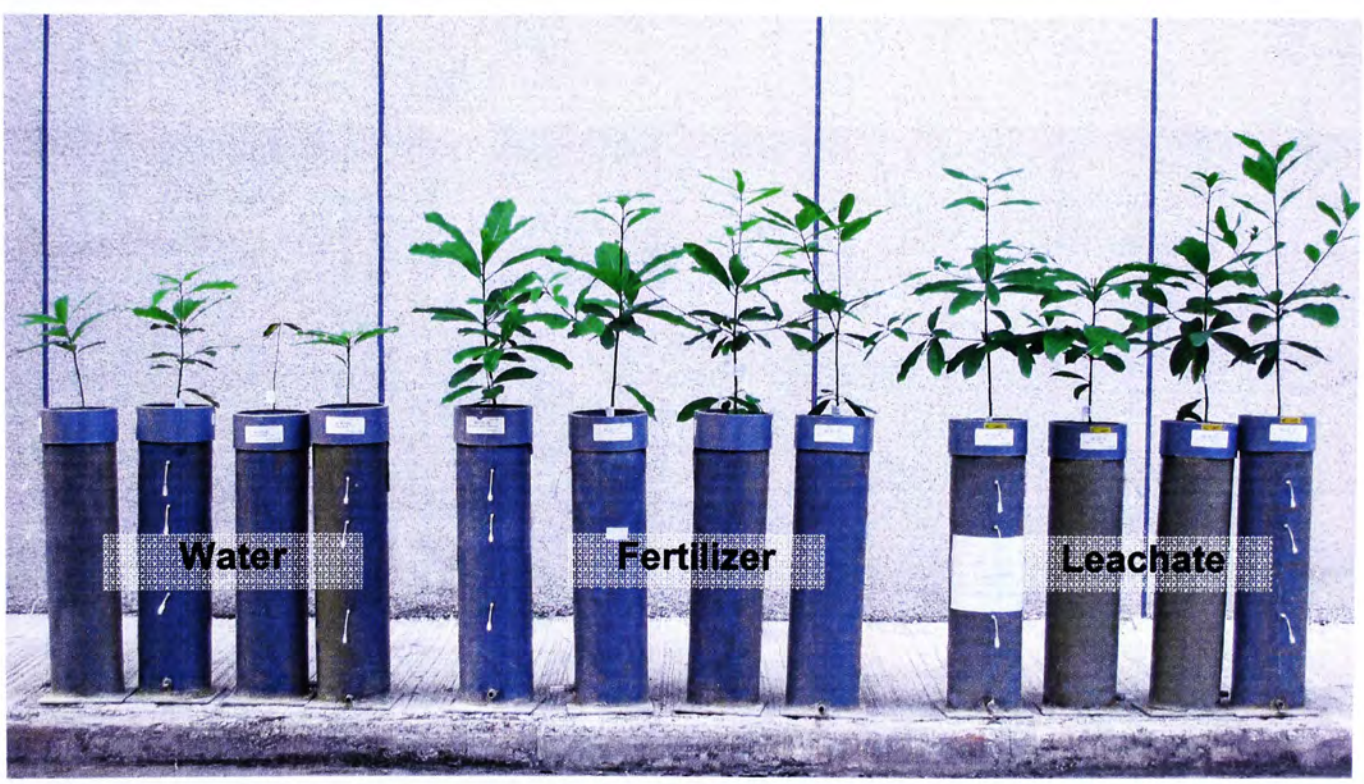


Plate 4.3 *Litsea glutinosa* after receiving water irrigation, fertilizer with water irrigation and leachate application for 12 weeks.

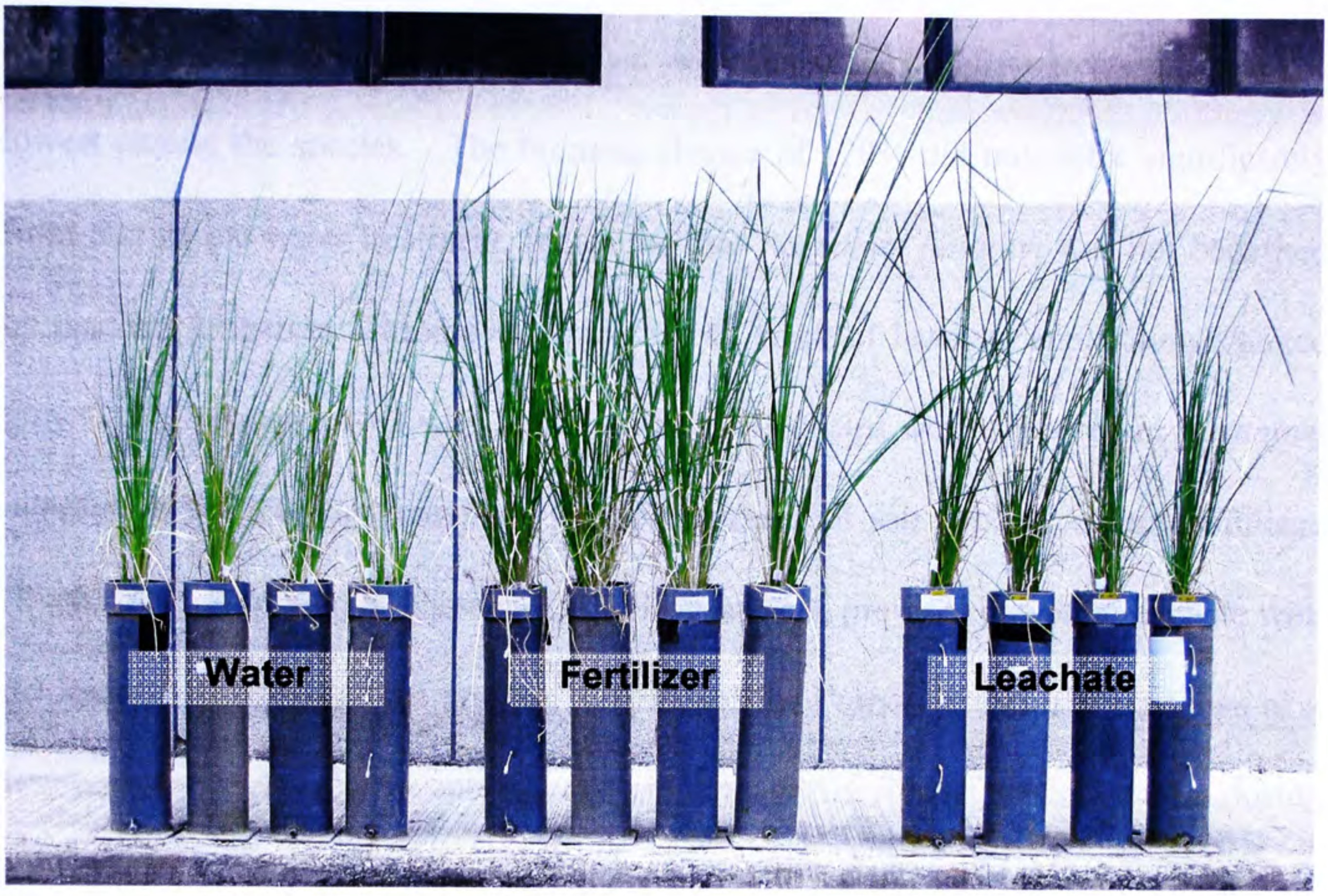


Plate 4.4 *Vetiveria zizanioides* after receiving water irrigation, fertilizer with water irrigation and leachate application for 12 weeks.

Plate 4.5 *Paspalum notatum* after receiving water irrigation, fertilizer with water irrigation and leachate application for 12 weeks.

However, the biomass gain of *Paspalum notatum* in the leachate treatment was the lowest among the species. The biomass change of 120% did not differ significantly from that of the water treatment, indicating that *Paspalum notatum* was not benefited by leachate irrigation. Moreover, since the 4th week of leachate application, stunted growth and narrow leaves with yellow or brown leaf tips were observed in *Paspalum notatum* in the leachate treatment, possibly because of salt deposition on the foliage (Plate 4.6). Applying leachate on the soil surface can prevent contact of leachate with the foliage on trees and tall grasses. However, for *Paspalum notatum* seedlings of a few centimeters in height, some tillers were temporarily submerged in leachate during irrigation. Subsequent evaporation of moisture leaded to high salt concentration on leaves and damage to foliage tissue. It seems that leachate applied by overland flow or spray irrigation is not desirable for the early growth of grass seedlings. Subsurface application may be a better alternative.

The stress symptoms of *Paspalum notatum* also suggested that, although germination tests using *Brassica chinensis* and *Lolium perenne* have been used in the bioassay of many environmental samples, they might not be the most suitable surrogate plants for the determination of leachate application rate. Germination tests, using the seeds of recipient plant species, may better reflect their sensitivity to leachate.

4.3.2.2 Tissue N contents

All plants in the leachate treatments had N contents higher than their respective control (Table 4.2). This finding was consistent with the finding in the previous

Table 4.2 Tissue N content, before and after 12 weeks of receiving application of diluted leachate and fertilizer.

Species	Treatment	Tissue N content (% w/w)	
		Aboveground	Underground
Hibiscus tiliaceus	Initial	1.19 ± 0.52	0.85 ± 0.37
	Water	1.20 ± 0.34 b	0.90 ± 0.26 b
	Fertilizer	2.20 ± 0.71 b	1.63 ± 0.50 b*
	Leachate	5.00 ± 0.50 a*	3.45 ± 0.36 a*
Litsea glutinosa	Initial	2.11 ± 0.09	1.62 ± 0.05
	Water	2.10 ± 0.20 c	1.55 ± 0.18 c
	Fertilizer	3.17 ± 0.32 b*	2.14 ± 0.22 b*
	Leachate	4.90 ± 0.58 a*	3.51 ± 0.38 a*
Paspalum notatum	Initial	1.87 ± 0.13	1.33 ± 0.05
	Water	1.93 ± 0.36 b	1.44 ± 0.19 b
	Fertilizer	1.76 ± 0.03 b	1.31 ± 0.12 b
	Leachate	3.90 ± 0.48 a*	2.75 ± 0.29 a*
Vetiveria zizanioides	Initial	1.16 ± 0.36	0.91 ± 0.31
	Water	0.79 ± 0.06 b	0.63 ± 0.12 b
	Fertilizer	1.68 ± 0.32 a	1.25 ± 0.31 ab
	Leachate	1.90 ± 0.62 a	1.34 ± 0.44 a

When compared within a species, values in a column with the same letter do not differ significantly at $P > 0.05$ by Tukey's test.
* Significantly different ($P < 0.05$) from the initial level by Student's t-test.

Plate 4.6 *Paspalum notatum* after receiving irrigation of diluted leachate for 30 days. Stunted growth, yellow or brown leaf tips were observed.

Plate 4.7 *Paspalum notatum* after receiving water irrigation for 30 days.

Experiment (Chapter 3). However, for plants receiving fertilizer application, *Hibiscus titiaceus* and *Paspalum notatum* did not exhibit elevation in their tissue N content, which can be attributed to the dilution by growth. The amount of N applied with fertilizer was limited. A transient increase in the available N in soil promoted plant growth in the first few weeks. However, soil N was gradually depleted by plant uptake and, more importantly, was lost by leaching loss. The levels of available NO_3^- and NHx declined to a level equal or even lower than the initial soil N contents. With the scarcity of available N in soil, N in older leaves would be translocated to growing parts to support their growth and diluted the tissue content in the whole plant (Tamm, 1991; Smith and Loneragan, 1997). In contrast, the amount of NHx in the leachate treatment was kept to in excess because of continuous addition of leachate N. Re-translocation of N within the plant might not be necessary.

Plants play an important role in the accumulation of soil N during ecological succession. The N originated from symbiotic N fixation and atmospheric deposition was taken up and stored in biomass. It is then returned to the soil N capital with litter fall (Rowell, 1996). The process is accelerated when there is more biomass formed with higher N content, in each growing season.

4.3.3 Soil and soil percolate

4.3.3.1 Percolate volume and soil moisture

Water input was a controlled variable in this study. Each soil column received on identical amount (24 x 3 mm) of tap water or diluted leachate each week. However, the amount of column percolate produced (Figure 4.3) varied with the treatment groups

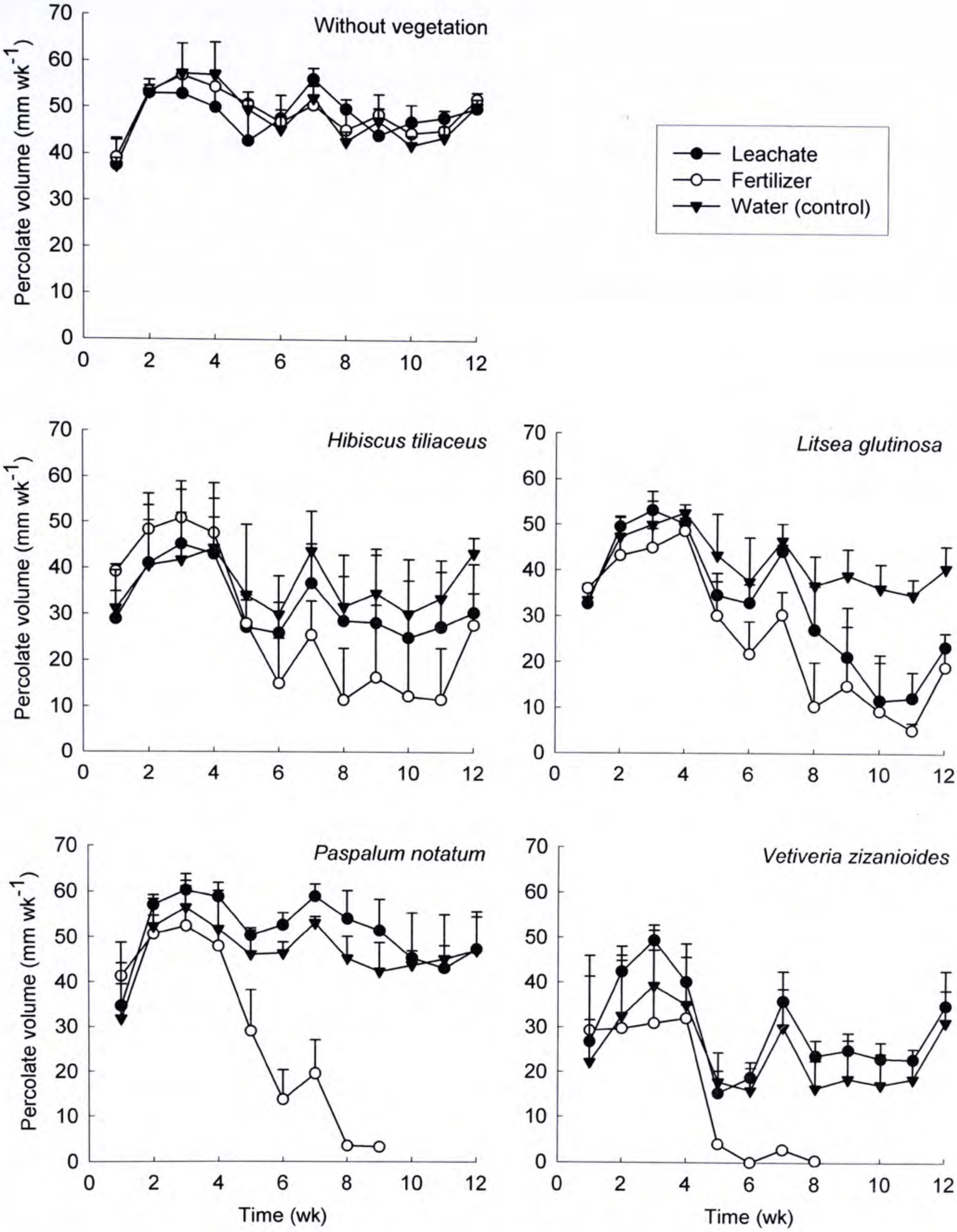


Figure 4.3 Temporal variation in the volume of column percolates, during 12 week irrigation with water (▲), water with fertilizer application (■) and diluted leachate (●). Error bars show the standard deviation (only the upper bar is shown) of 4 replicates.

and the types of vegetative cover which affected the evapotranspiration from the soil-plant systems. In the columns without vegetation, the percolate volume changed within a narrow range of 45 - 60 mm week^{-1}. Treatments with leachate and fertilizer did not lead to a significant difference in percolate volume. On this basis, about 20 - 40% of applied water was lost by evaporation, equivalent to 1.57 - 3.13 mm day^{-1}. This soil evaporation rate was slightly lower than the ambient values at the same period in Hong Kong (Figure 4.4). This can be attributed to the higher humidity in the greenhouse and good drainage of the growth media. The sandy loam drained water to lower horizons rapidly and reduced evaporation from the soil surface.

The decrease in the ambient solar radiation and evaporation during the 6th and 7th week (Figure 4.4) resulted in higher percolate volume collected in the 7th week. A transient increase in the percolate volume was also observed in all columns regardless of treatment and vegetation. Columns with vegetation in general produced smaller amounts of percolate. The percolate volume gradually decreased with time because of the increased uptake as plants grew. When compared within plant species, columns receiving fertilizer produced the least amount of percolate. The two herbaceous species were much more effective in reducing the percolate volume in the fertilizer treatment as no column percolate was produced from the 10th week onwards. Although leachate application promoted plant growth, only the leachate treatments in *Hibiscus tiliaceus* and *Litsea glutinosa* produced smaller volume of column percolate compared with the control. The percolate volume generated by the leachate-treated *Paspalum notatum* and *Vetiveria zizanioides* was slightly higher than the control throughout the experiment.

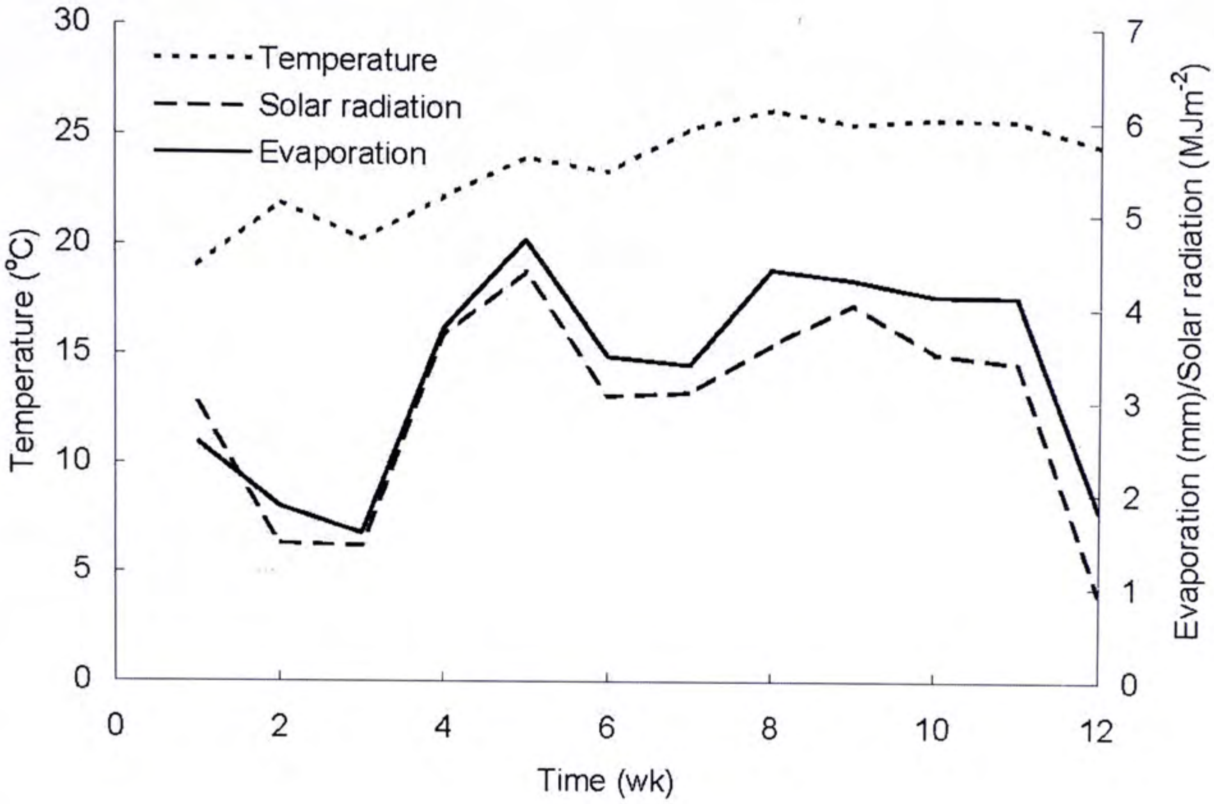


Figure 4.4 Air temperature, evaporation and solar radiation in Hong Kong during the experimental period. The time axis is the number of weeks counted from the first leachate application (data from Hong Kong Observatory, 2003c).

Research shows water depletion by increased N application (Ottman and Pope, 2000), through its influence on the amount and duration of crop growth. However, research has not been conclusive about the effect of leachate application on plant transpiration. At least two interacting factors determine the transpiration rate of leachate-treated plants. An increase in the total leaf area, in response to the nutrient supply, led to a higher transpiration rate (Ettala, 1987). Moreover, factors affecting stomatal opening also affect evapotranspiration rate per unit leaf area. In dry seasons, application with diluted or low strength leachate can relieve the water stress and thus increase the stomatal conductance for water vapor (Liang *et al.*, 1999). On the other hand, leachate-treated plants may suffer from osmotic stress as a result of increased soil salt content. Sensitive species may exhibit a reduction in stomatal conductance (Cureton *et al.*, 1991; Stephens *et al.*, 2000). The total evapotranspiration rate from the whole plant is suppressed even when foliage area increased.

Change in the volume of soil percolate can affect the movement of ions as well as their concentrations in soil. In leachate irrigated sites, increased transpiration can improve the stability of cover soil by reducing the hydraulic loading. However, excessive transpiration may slow down leaching, resulting in salt accumulation in the soil. If this happens, alternating with water irrigation may be required.

4.3.3.2 pH

Unlike other studies on leachate irrigation, a decrease in the soil pH was observed in this experiment. When compared within the vegetation type, columns receiving leachate had the lowest soil pH. The extent of this decrease varied with the types of vegetative cover. However, vertical variation in soil pH and a noticeable change in the pH of percolate were not observed in all treatment groups, possibly because of the buffer of residual acidity of soil.

Nitrification is an acidifying process, which liberate H^+. The final change in soil pH also depend on plant uptake and leaching of the NO_3^- originating from nitrification. When NO_3^- is formed and is rapidly taken up by roots and reduced to NH_4^+, HCO^- ion would be released to balance the electrical neutrality between cell membranes. There is no net change in soil acidity as the HCO^- neutralized the H^+ produced in nitrification. However, the two processes may be decoupled. If NO_3^- uptake lags behind nitrification, as would be the case in N saturation, soil may be acidified (Tamm, 1991). Acidification becomes more persistent as the NO_3^- is leached and is not longer available for root uptake. The effect of plant uptake of NO_3^- on soil acidification can be observed when comparing the change in soil pH and the total amount of NOx-N in column percolates (Table 4.5, in Section 4.3.4.2).

The soil for packing the columns was slightly acidic initially, with a pH of 5.92 ± 0.44 (Figure 4.5). In the columns without vegetative cover, there was no plant uptake to counteract the acidification of nitrification. Leaching loss of NO_3^- in the leachate treatment was 199 kg N ha^{-1}, and the pH dropped to 4.28 ± 0.19. Similarly, leaching of 225 kg N ha^{-1} in the leachate treatment of *Paspalum notatum* resulted in a final soil pH of 3.87 ± 0.12. In contrast, soil planted with *Vetiveria zizanioides* had a lower

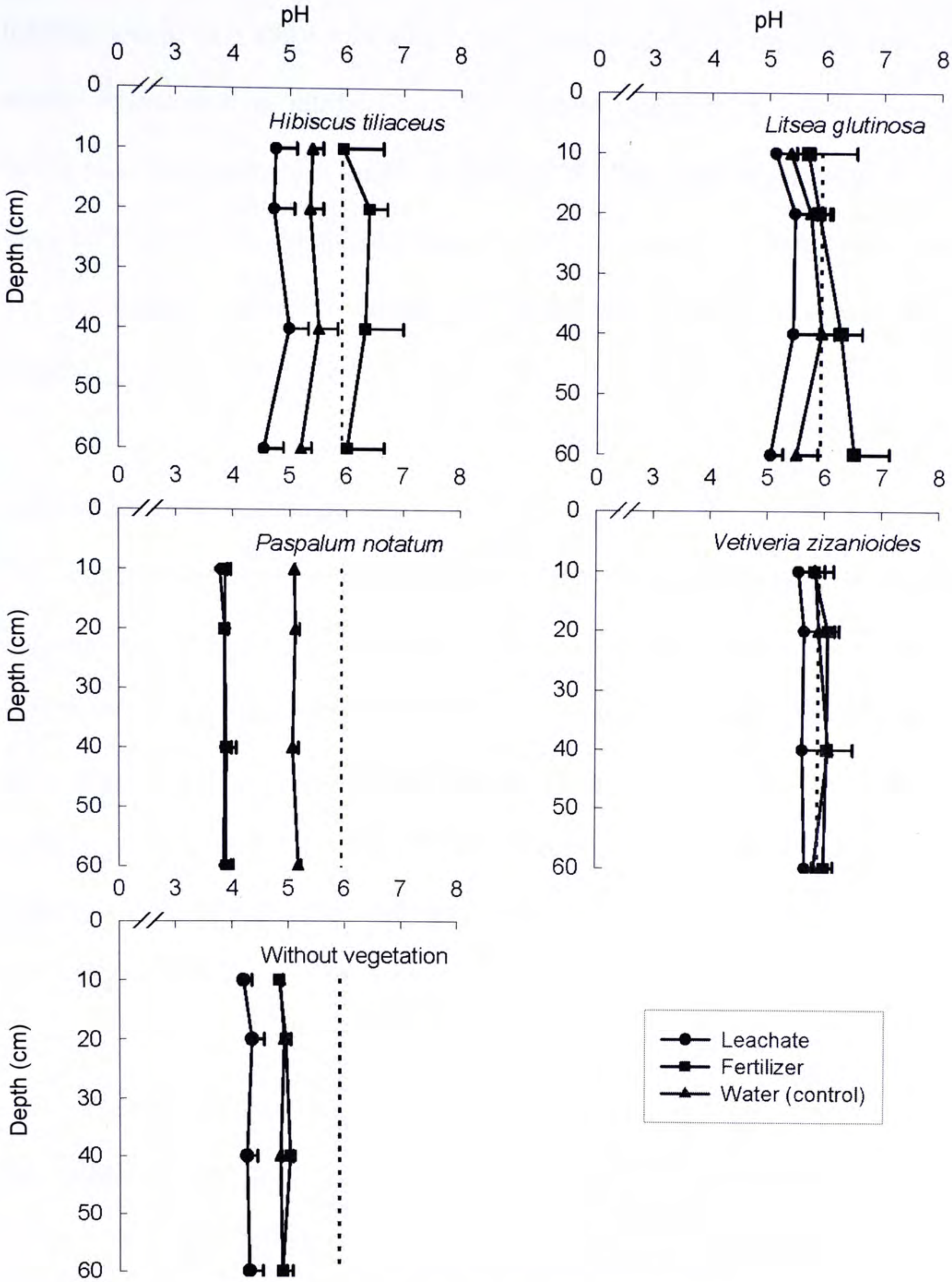


Figure 4.5 Soil pH at different depths, after 12-week irrigation with water (▲), water with fertilizer application (■) and diluted leachate (●). Broken lines indicate the initial pH level. Error bars show the standard deviation (only the upper bar is shown) of 4 replicates.

leaching loss of NO_3^- (90.4 ± 21.4 kg N ha^{-1}), as root uptake neutralized a part of the acidity originated from nitrification. The leachate treatment of vetiver grass only exhibited a slight decrease in soil pH to 5.64 ± 0.28. The order of change in pH in the leachate treatment was *Paspalum notatum* = without vegetation > *Hibiscus tiliaceus* > *Litsea glutinosa* > *Vetiveria zizanioides*, which was consistent with the order of leaching loss of NO_3^- (Table 4.5).

4.3.3.3 Electrical conductivity

Electrical conductivity (EC) not only reflects the salt content in soils; but also indicates the front of ion movement. Increase in soil salintiy is one of the environmental concerns in leachate irrigation. The previous chapter has discussed the cumulative effects of leachate irrigation on the soil EC and the use of leaching requirement to control the levels of salts (Chapter 3). This section focuses on the effects of different treatments and vegetative cover on the EC of soils and percolates (Figures 4.6 and 4.7).

The electrical conductivity of tap water ranged between 36 - 300 μS cm^{-1}, with an average of 150 μS cm^{-1} (WSD, 2004). With the initial soil EC of only 32.0 ± 1.41 μS cm^{-1}, the granitic soil did not add much salt to the percolate. In the water (control) treatments, the EC of percolates fluctuated within a narrow range of 187 - 323 μS cm^{-1} (Figure 4.6). Differences between vegetation types were not observed.

Since the 3rd week, the percolate EC of the leachate treatments began to increase at the rate of 209 - 346 μS cm^{-1} per week (equivalent to 134 - 221 mg L^{-1} TDS).

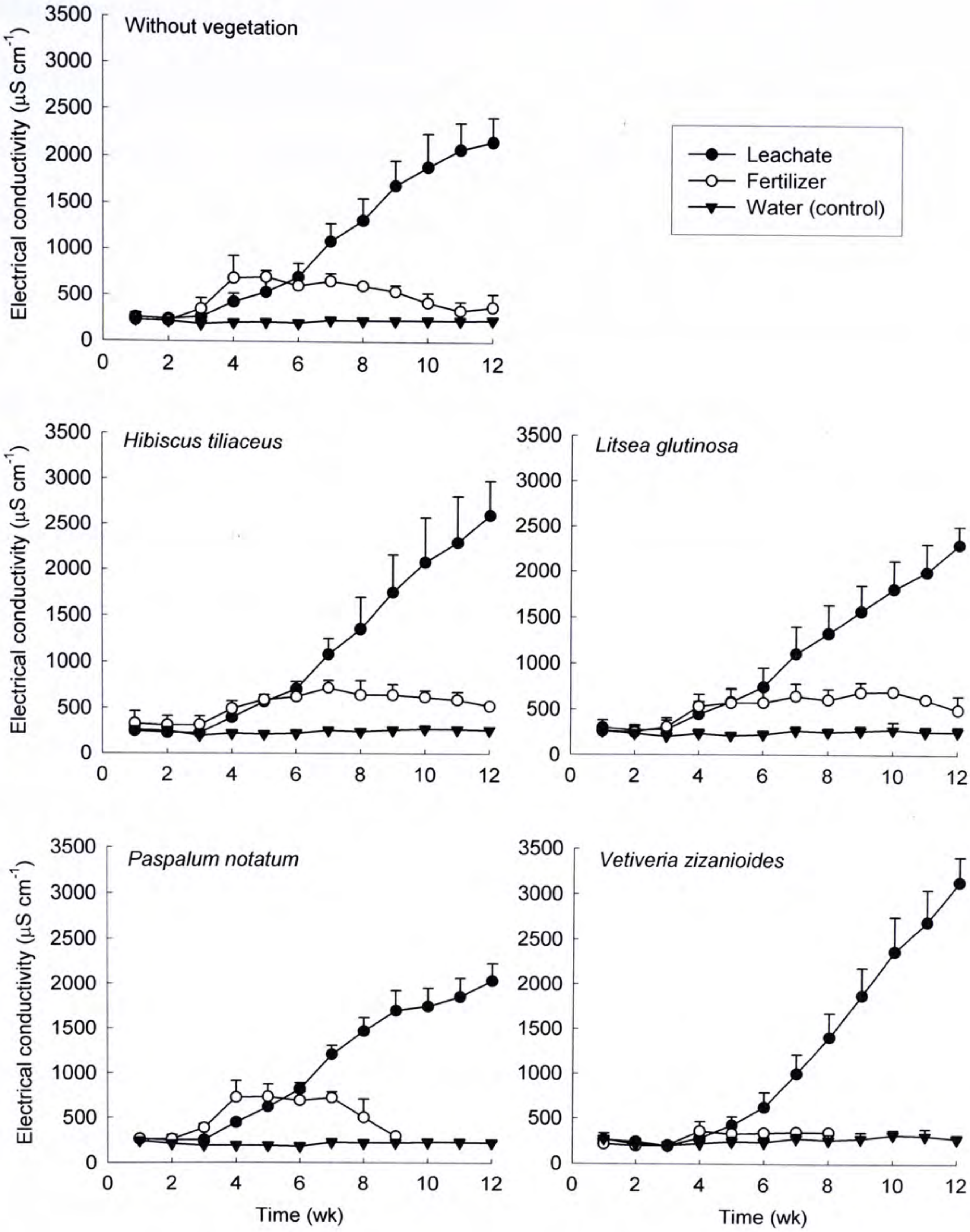


Figure 4.6 Temporal variation in the electrical conductivity of soil percolate, during 12-week irrigation with water (▲), water with fertilizer application (■) and diluted leachate (●). Error bars show the standard deviation (only the upper bar is shown) of 4 replicates. There was no EC reading in the fertilizer treatment of *Paspalum notatum* and *Vetiveria zizanioides*, after the 9^{th} and 8^{th} weeks, since the volume of percolate was too small for the measurement.

The trend continued to the end of the experiment. The order of increment was *Vetiveria zizanioides* >> *Hibiscus tiliaceus* > *Litsea glutinosa* > *Paspalum notatum* = without vegetation. The differences can be attributed to transpiration which reduced the volume of percolate and thus produced percolates of higher ion concentration.

In the fertilizer treatment without vegetation, the EC of the percolate also increased slightly since the 3rd week, indicating the leaching of salts such as NO_3^- PO_4^{3-}, Cl^-, K^+ and NH_4^+. The percolate EC came to a plateau at 600 μS cm^{-1} and gradually decreased to the baseline value of about 300 μS cm^{-1}. Similar trends were observed in the columns with plants, with the exception of vetiver grass. In the columns with vetiver grass, the leaching loss was limited by the rapid uptake of water and nutrients. The percolate EC was lower than in the fertilizer treatment with other plant species, which remained steady at about 350 μS cm^{-1} until no column percolate was collected since the 8th week.

When compared within species, the soil EC of the leachate treatment was the highest (Figure 4.7). In the columns without vegetation, the soil EC of the leachate treatment increased by 7 times, reaching 2550 μS cm^{-1} in the 12th week. High transpiration rate in vetiver grass led to the increase in percolate EC. The soil EC of vetiver grass with leachate irrigation was found to be the highest among the vegetation types. Fortunately, the soil EC was far below the threshold level of vetiver (8 mS cm^{-1}) (Truong, 1994). The watering rate was high enough to keep the soil Cl^- at a safe level. However, the leaching requirement of areas planted with vetiver grass may not be met, owing to high transpiration rates. Special attention should be paid to the change in

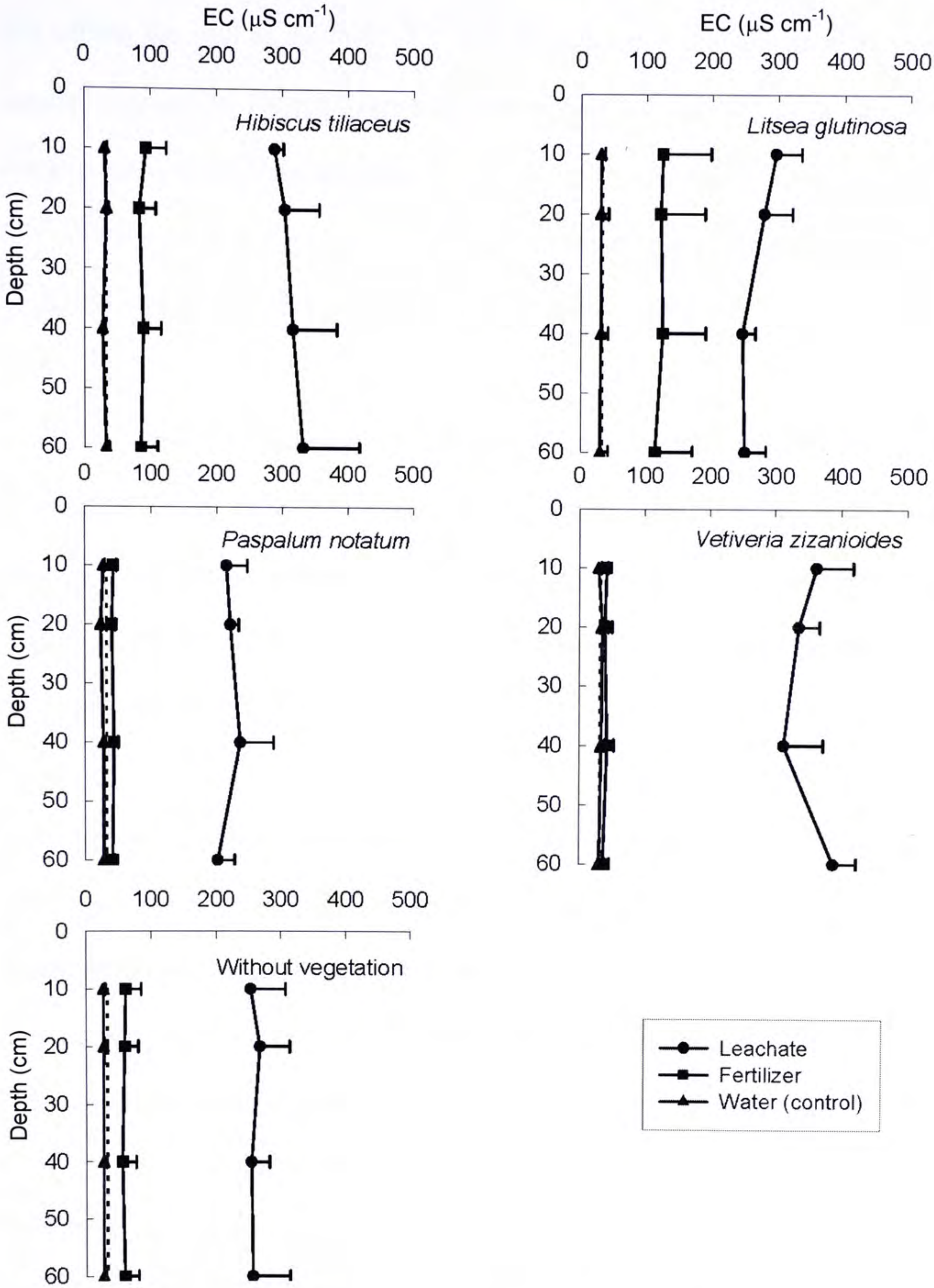


Figure 4.7 Levels of the electrical conductivity of soil extract at different soil depths, after 12-week irrigation with water (▲), water with fertilizer application (■) and diluted leachate (●). Broken lines indicate the initial EC level. Error bars show the standard deviation (only the upper bar is shown) of 4 replicates.

soil salinity (as well as the level of Cl^-) in dry seasons. Trimming vetiver grass at regular intervals to control evapotranspiration may be required. The amount of leaching seems to be adequate to keep the soil EC in a reasonable range. Owing to the low salt contents in the soil and irrigation water, there was no net change in the soil salt content in the corresponding treatments after 12 weeks of irrigation.

The addition of fertilizer only led to a slight increase in soil EC (Figure 4.7). The soil EC in the fertilizer treatments did not exceed 100 $\mu S\ cm^{-1}$ most of the time. The soil EC of columns planted with *Hibiscus tiliaceus* and *Litsea glutinosa* was higher than those without vegetation, which can be attributed to the reduced leaching loss in the vegetated columns.

On the other hand, plant uptake could reduce soil salt contents. For example, the rapid growth of *Paspalum notatum* and *Vetiveria zizanioides* in the fertilizer treatment increased their demand for mineral nutrients in soil. When the nutrient ions applied with fertilizer were lost in plant uptake and leaching, the soil EC of two treatment groups declined to the baseline level.

4.3.3.4 Nitrate

NO_3^- is the primary form of N leaching from soil (Rowell, 1996; Snyder, 1996). It is a negatively charged ion that is repelled, rather than attracted, to the cation exchange sites on clay particles. NO_3^- is completely soluble at any concentration found in soil and can move readily with percolating water. Owing to the low retention of NO_3^- in soil, the initial level of NOx-N in soil was as low as 5.90 ± 1.98 mg kg^{-1}.

Irrigation water with an average NOx-N concentration of 7.4 mg L^{-1} (WSD, 2004) did not increase the level of soil NO_3^-, but removed it by leaching. In the control, the level of NO_3^- in percolate was kept at a low level of < 5 mg L^{-1}, sometimes below 0.2 mg L^{-1} (Figure 4.8).

Application of fertilizer led to a transient increase in soil NO_3^-. However, without vegetation, leaching loss of NO_3^- was observed since the 2nd week, peaked at 45.3 mg L^{-1} in the 5th week and gradually decreased as soil NO_3^- was depleted (Figure 4.8). Plant uptake reduced NO_3^- leaching in the fertilizer treatment. In columns planted with *Vetiveria zizanioides*, the peak NO_3^- concentration in percolate was only 15 mg L^{-1}, which was one-third of that measured in the columns without vegetation. Prolonged leaching loss, together with plant uptake, depleted the NO_3^- applied with fertilizer, or even the existing N reserve in the soil. The soil NO_3^- content of the fertilizer treatment was lower than that of the initial level (Figure 4.9), regardless of the vegetation type.

Raw leachate contained small amounts of NOx-N (< 0.2 mg L^{-1}). However, soil irrigated with diluted leachate exhibited drastic increases in the levels of NO_3^- (Figure 4.9), which can be attributed to the nitrification of NHx. After leachate irrigation, the amount of NOx-N in the soil was over 10 times the initial level. There were no significant differences in the NO_3^- contents among soils planted with different plant species. Plant uptake would have a small influence on the residual NO_3^- when the available N was in large excess to the nutrient requirement.

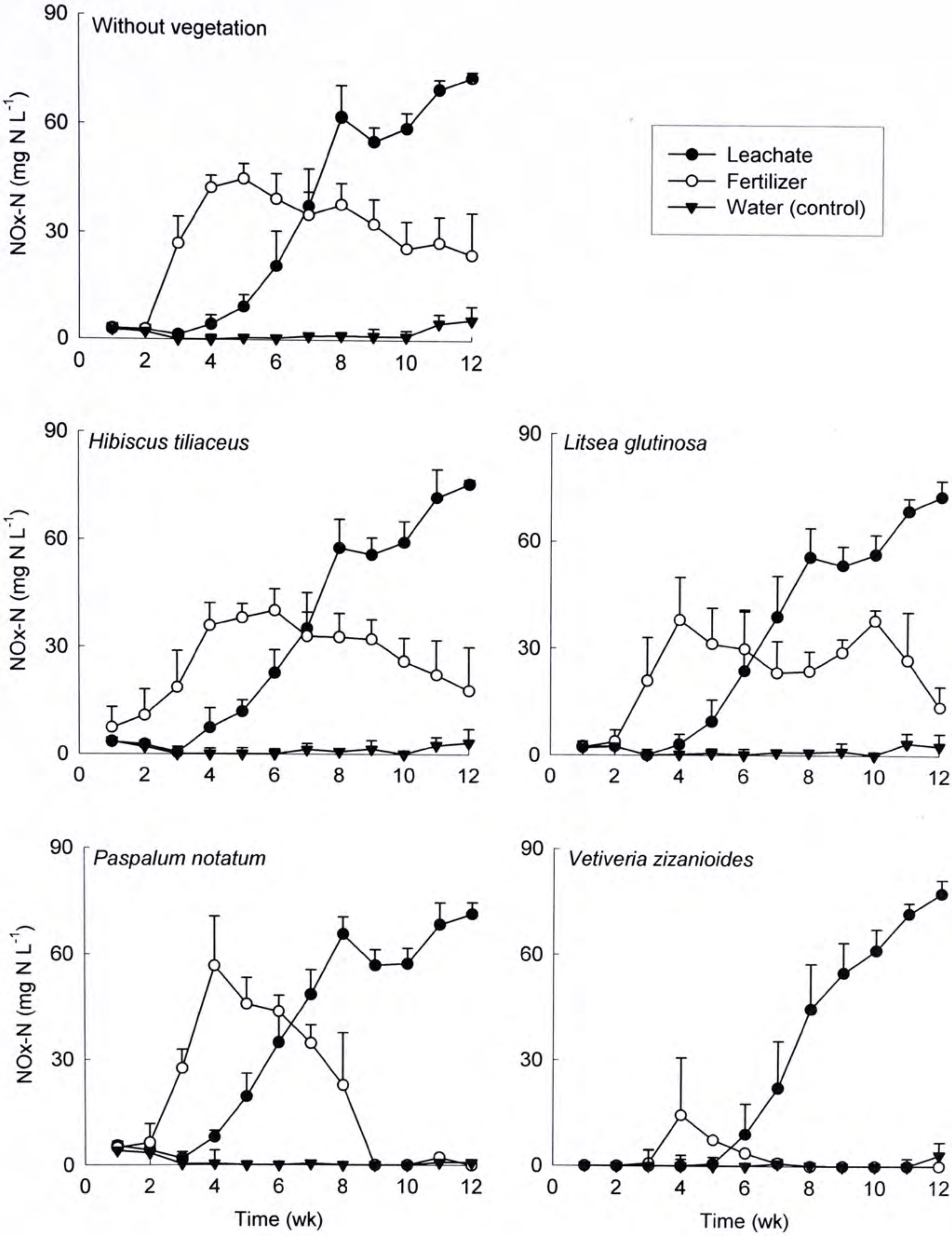


Figure 4.8 Temporal variation in the level of NOx-N in soil percolates, during 12-week irrigation with water (▲), water with fertilizer application (■) and diluted leachate (●). Error bars show the standard deviation (only the upper bar is shown) of 4 replicates.

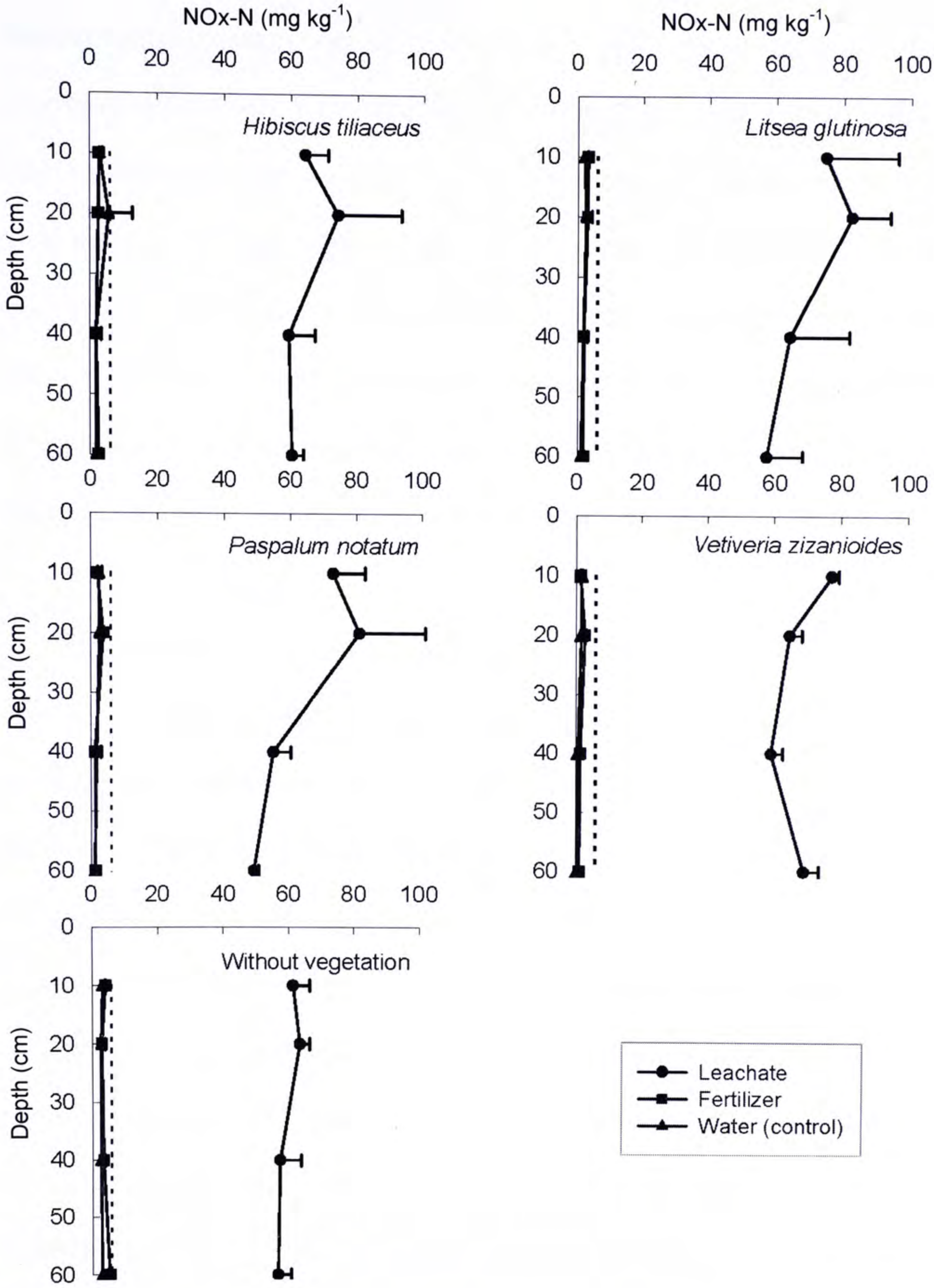


Figure 4.9 Levels of NOx-N in soil at different depths, after 12-week irrigation with water (▲), water with fertilizer application (■) and diluted leachate (●). Broken lines indicate the initial NOx-N level. Error bars show the standard deviation (only the upper bar is shown) of 4 replicates.

Compared with the fertilizer treatment, the onset of NO_3^- leaching from the leachate-treated columns was delayed by 1 - 2 weeks, depending on the type of vegetative cover (Figure 4.8). The lag time may be attributed to the time required for nitrification and the proliferation of the nitrifier community. Nitrifiers are favoured by good soil aeration and ample supply of NHx and organic carbon. The rate of nitrification increased to a detectable level since the 5^{th} week and reached a plateau at about 60 mg N L^{-1}. The rate limiting factor was not known. The concurrent increase in percolate NHx-N concentration suggested the inhibition of nitrification by the increasing amount of free NH_3 in soil moisture (Stevenson and Cole, 1999).

Low residual NO_3^- in soil suggested a drawback of fertilizer over leachate. Fertilizer is readily available in most areas. Application is possible in almost all situations and practitioners need not worry about the phytotoxic effects of artificial fertilizer. However, it is impossible to provide the annual nutrient requirement in a single dose on newly restored sites. In the absence of established vegetation, a large proportion of the mineral N is lost by leaching. A large dose of N, say 200 kg N ha^{-1} in this study, might promote plant growth initially. However, when the available N is depleted by leaching and plant uptake, the vegetation may suffer from N deficiency eventually. Bradshaw (2002) suggested an application rate of 50 kg N ha^{-1} each time to reduce the leaching loss. However, repeated applications have to be made each year with an allowance for leaching losses.

In contrast, leachate irrigation provided a stable supply of N in a readily available form. It can be applied with irrigation water. The concentration of leachate can be adjusted according to the nutrient requirement of plants and at the same time prevent substantial leaching loss.

4.3.3.5 Ammonium

Ammonicial nitrogen (NHx-N) contributed over 84% of the total Kjeldahl nitrogen (TKN) in the leachate samples. Therefore, the fate and distribution of the NHx after leachate application are of special interest. The interaction of NHx with the soil-plant system is more complicated than the abovementioned anions. Firstly, considerable amounts are assimilated by soil microorganisms. Secondly, higher plants are able to use NHx; young plants are especially capable in this respect. Cationic NH_4^+ is capable of exchange with the CEC of soil, or of being fixed within clay lattices. It is susceptible to microbial transformation and volatilization. Such losses are significant when large quantities of NHx are added to the soil. When the needs of plants are temporarily satisfied, the remaining NHx may be readily oxidized in the nitrification process. Any NH_4^+ ions left in the soil solution may leave the soil with percolating water, but this is seldom an important pathway in natural ecosystems.

In the fertilizer and the control treatments, the percolate NHx-N remained at a low level of < 1 mg L^{-1} (Figure 4.10). Only the columns in the leachate treatment showed elevated NHx-N concentration in the percolate, possibly because of the high N application rate. The percolate NHx-N concentration began to rise since the 7[th] week, after receiving percolation with 1.67 pore volumes of diluted leachate. Compared with NO_3^- concentration, the onset of NHx leaching was delayed for about 3 weeks

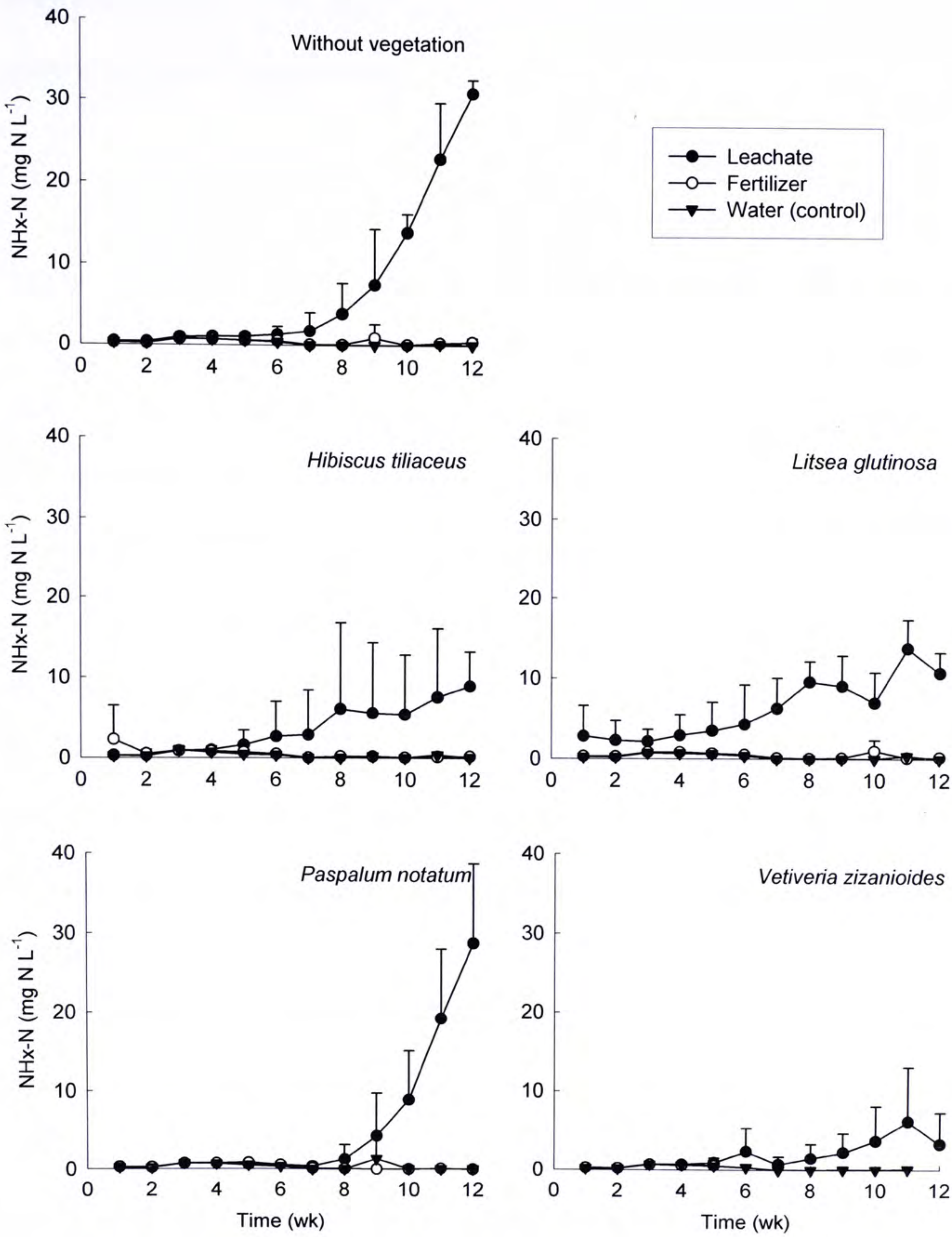


Figure 4.10 Temporal variation in the level of NHx-N in soil percolates, during 12-week irrigation with water (▲), water with fertilizer application (■) and diluted leachate (●). Error bars show the standard deviation (only the upper bar is shown) of 4 replicates.

(0.63 pore volumes). The delay can be attributed to cation exchange, NH_4^+ fixation, nitrification and NH_3 volatilization.

It has long been known that many soils are capable of retaining considerable amounts of NH_4^+ in non-exchangeable forms. An average of 10% of the N in mineral soils was assumed to occur as clay-fixed NH_4^+ (Rowell, 1996; Stevenson and Cole, 1999). Fixation is the result of substitution of NH_4^+ for interlayer cations (Na^+, Ca^{2+} and Mg^{2+}) within the lattice of clay minerals. NH_4^+ ions with diameters of about 2.8 nm are capable of fitting into the voids between lattice and thereby becoming trapped, or fixed as an integral part of the lattice structure. When occupied by a cation, the lattice layers contract and are bound together electostatistically, thereby preventing the entrance of cations with diameters greater than 2.8 nm, such as Na^+, Ca^{2+} and Mg^{2+}. NH_4^+ fixation, cation exchange and volatilization reduced the amount of mobile NHx in soil. The capacity was saturated eventually by ample supply of NHx from leachate, leading to an increase in the NHx in soil pore water. Moreover, as the nitrification activity increased in response to the elevated concentration of NHx, considerable amounts of NHx were transformed and leached in the form of NO_3^-. NH_4^+ fixation, volatilization and cation exchange, together with the uptake by plant and nitrifying bacteria reduced the amount of mobile NHx in soil pore water. Leaching of NHx was kept to a minimal in the first few weeks. The capacity was saturated eventually by ample supply of NHx from leachate, and surplus NHx leached out in the soil percolate.

Vegetative cover had a profound effect on the amount of NHx in the percolate. When compared with the leachate treatments of different plant species, columns planted with *Hibiscus tiliaceus, Litsea glutinosa* and *Vetiveria zizanioides* had percolate NHx-N concentrations below 10 mg L^{-1} throughout the course of leachate irrigation. In contrast, in the column planted with *Paspalum notatum*, the concentration of percolate NHx-N was similar to that from the columns without vegetation, possibly because of the suboptimal growth of *Paspalum notatum* in the leachate treatment.

Uptake of NHx for vegetative growth not only limited the amount of NHx leaving the soil-plant system, but also affected the amount of residual NHx in soil (Figure 4.11). Leachate treatment led to a remarkable increase in the soil NHx, except in columns with *Vetiveria zizanioides*, where the soil NHx-N content was below 20 mg kg^{-1}. The fine, massive root system of *Vetiveria zizanioides* might have facilitated the uptake and volatilization of NH_3 from soil. Similarly, soil planted with *Hibiscus tiliaceus* and *Litsea glutinosa* also had increased soil NHx after leachate application, but the NHx content was only two-thirds of that in the columns without vegetation.

In addition, vertical variation in soil NHx was observed in the leachate treatments (Figure 4.11). The highest level of soil NHx was found at the 40 cm depth. The movement of NHx in soil is limited due to retention by exchange sites, NH_4^+ fixation by clay and root uptake. The depth of NHx movement can vary with soil type, the volume of precipitation, N application rate and the presence of vegetative cover. It is generally believed that clayey soil with a high CEC under low precipitation conditions

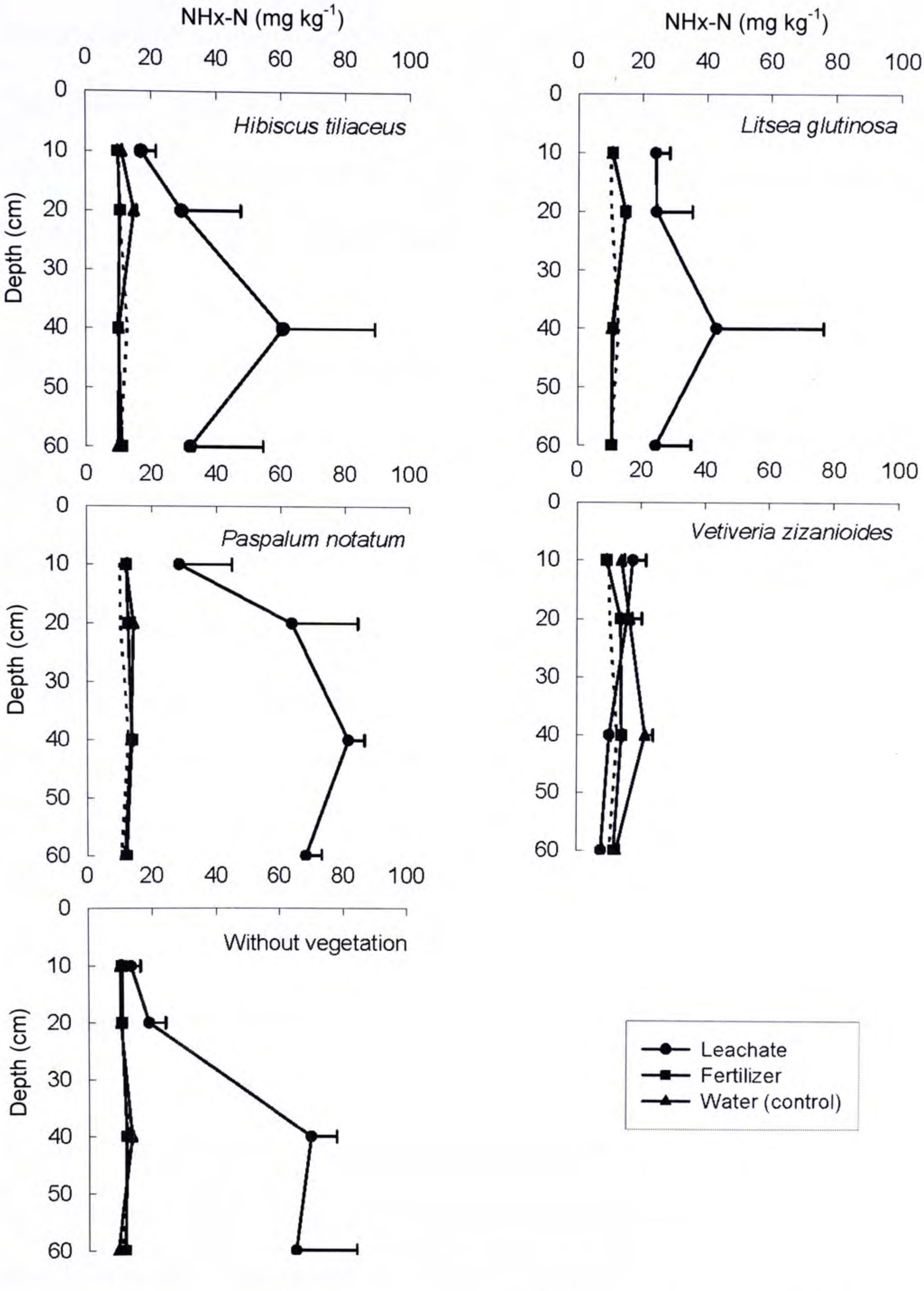


Figure 4.11 Levels of NHx-N in soil at different depths, after 12-week irrigation with water (▲), water with fertilizer application (■) and diluted leachate (●). Broken lines indicate the initial NHx-N level. Error bars show the standard deviation (only the upper bar is shown) of 4 replicates.

retains NH_4^+ better. Studies on different soil types have shown that most of the NHx was retained within the upper 50 cm of soil (Cai *et al.*, 1985; Sun, 1987; Ottman and Pope, 2000). Since increasing amounts of NHx were found in the soil percolate, the vertical variation in the soil NHx would get smaller as the soil capacity was saturated by the large influx of NHx from leachate.

4.3.4 N balance of the soil-plant system

To rebuild the N capital without causing secondary environmental problems, a fundamental understanding of the processes involved in the accumulation of N is required. Gain in soil N occurs by symbiotic N fixation and by the addition of NHx and NO_3^- from precipitation; losses take place by biomass removal, leaching and volatilization. Labile forms are assimilated and immobilized into organic N by plants and microbes. Organic N in debris is subject to mineralization by microbial and chemical processes. NHx is oxidized into NO_3^- in favourable conditions. NO_3^- is ultimately returned to the atmosphere as molecular N_2 by biological denitrification, thereby completing the cycle. N is accumulated in the ecosystem when the rate of gain exceeds the rate of mineralization and loss.

4.3.4.1 Change in the N capital after leachate irrigation

The existing soil N capital consisted of the total amounts of N in soil and plant biomass measured before leachate application, regardless of their chemical forms. In natural ecosystems, the major fraction of the N capital is not available to plant uptake. It is gradually released by mineralization of organic matter. The size of the N capital affects the amounts of N made available per unit of soil per unit of time and consequently determines the types of ecosystem that can be supported (Ingestad, 1982). Keeping the mineralization rate unchanged, a higher N flux density can support more plants as well as the growth of some nutrient-demanding plants. Tree seedlings or saplings cannot survive and develop to mature trees unless they have a higher relative growth rate than older trees and consequently they need a higher N flux density. When organic matter is mineralized at the rate of 1 - 10%, a self-sustaining vegetation cover in temperate regions requires a minimal soil N reserve/capital of 1000 kg N ha^{-1} and a further amount for the capital contained in the vegetation itself to provide an annual N influx of 200 kg N ha^{-1}.

In the present study, the soil N capital of 1250 - 1330 kg N ha^{-1} was marginal (Table 4.3). Owing to the low amount of N stored in biomass, the soil N capital was susceptible to depletion as trees grew. The amounts of NHx and NO_3^- were also too low to support the immediate nutrient requirement in the growing season. To accelerate the establishment of vegetative cover, external supply of mineral N is recommended to support the early growth of trees and grass seedlings.

It was anticipated that application of leachate and fertilizer would increase the soil N capital. However, the present study showed that the resulting N capital depended on more factors than NHx influx alone. In the columns without vegetation, the control and fertilizer treatments exhibited a decrease in the N capital (Table 4.4). In the control, leaching led to the decrease of TKN, from 1150 to 550 kg N ha^{-1}. Leaching loss of NO_3^- was evident, with the NOx-N reduced by 50% after water irrigation for 12 weeks. There was a slight increase in NHx-N, possibly because of

Table 4.3 Initial N capital and the external supply from leachate and fertilizer.

Vegetation type	Treatment	Initial biomass		Soil[1]			Initial N	External	Total
		Aboveground	Underground	NOx-N	NHx-N	TKN	capital	supply	input
Without vegetation	Water			45.2	58.3	1150	1250		1190
	Fertilizer			45.2	58.3	1150	1250	200	1390
	Leachate			45.2	58.3	1150	1250	1920	3110
Hibiscus tiliaceus	Water	15.3	10.2	45.2	58.3	1150	1280		1220
	Fertilizer	15.3	10.2	45.2	58.3	1150	1280	200	1420
	Leachate	15.3	10.2	45.2	58.3	1150	1280	1920	3130
Litsea glutinosa	Water	41.2	31.2	45.2	58.3	1150	1330		1260
	Fertilizer	41.2	31.2	45.2	58.3	1150	1330	200	1460
	Leachate	41.2	31.2	45.2	58.3	1150	1330	1920	3180
Paspalum notatum[2]	Water	17.0	28.4	45.2	58.3	1150	1300	20	1260
	Fertilizer	17.0	28.4	45.2	58.3	1150	1300	200 + 20	1460
	Leachate	17.0	28.4	45.2	58.3	1150	1300	1920 + 20	3170
Vetiveria zizanioides	Water	47.7	59.2	45.2	58.3	1150	1360		1300
	Fertilizer	47.7	59.2	45.2	58.3	1150	1360	200	1500
	Leachate	47.7	59.2	45.2	58.3	1150	1360	1920	3220

[1] The chemistry of soil used for preparing soil column

[2] 20 kg N ha^{-1} of fertilizer was applied to all treatment groups for the initial growth of *Paspalum notatum*.

All units in kg N ha^{-1} unless otherwise specified.

Table 4.4 Final N-capital (in soil and vegetative cover) at the end of the leachate irrigation experiment.

Vegetation type	Treatment	Biomass		Soil			Final N capital	Change
		Aboveground	Underground	capital	NHx-N	TKN		
Without vegetation	Water			22.1 ± 8.53	83.8 ± 8.91	550 ± 89.6	579 ± 86.4 b	-612± 86.4 b
	Fertilizer			30.8 ± 15.7	85.3 ± 10.5	634 ± 96.5	660 ± 123 b	-530± 123 b
	Leachate			452 ± 19.9	379 ± 38.4	844 ± 95.4	1300 ± 87.7 a	68.3± 57.3 a
Hibiscus tiliaceus	Water	38.4 ± 21.7	41.7 ± 19.4	20.0 ± 8.52	84.0 ± 9.2	269 ± 81.2	350 ± 62.8 c	-866± 62.8 c
	Fertilizer	163 ± 71.7	381 ± 159	17.9 ± 10.9	79.5 ± 12.3	640 ± 38.8	1170 ± 60.3 b	-42.2± 60.3 b
	Leachate	263 ± 39.9	559 ± 81.1	483 ± 25.8	320 ± 179	851 ± 161	2260 ± 288 a	1050± 288 a
Litsea glutinosa	Water	54.7 ± 26.2	23.5 ± 6.15	15.4 ± 8.46	87.3 ± 14.4	264 ± 22.8	350 ± 8.54 c	-866± 8.54 c
	Fertilizer	272 ± 14.7	165 ± 13.9	19.6 ± 2.47	87.8 ± 15.2	559 ± 69.4	1050 ± 32.9 b	-217± 32.9 b
	Leachate	387 ± 125	277 ± 95.3	511 ± 37.6	235 ± 147	960 ± 183	2180 ± 189 a	919± 189 a
Paspalum notatum	Water	20.5 ± 13.6	47.2 ± 25.6	13.2 ± 3.56	97.7 ± 14.9	746 ± 15.0	803 ± 64.7 b	-433± 64.7 b
	Fertilizer	210 ± 16.5	511 ± 64.0	18.4 ± 5.42	97.9 ± 8.62	802 ± 24.8	1510 ± 46.5 a	272± 46.5 a
	Leachate	80.2 ± 82.4	183 ± 200	462 ± 53.8	498 ± 46.6	1140 ± 61.1	1700 ± 169 a	469± 169 a
Vetiveria zizanioides	Water	91.3 ± 15.2	114 ± 28.1	6.69 ± 1.07	71.2 ± 6.31	262 ± 12.7	452 ± 74.4 b	-846± 74.4 b
	Fertilizer	507 ± 90.2	449 ± 107	11.5 ± 2.81	95.8 ± 24.0	855 ± 88.5	1820 ± 280 a	525± 280 a
	Leachate	295 ± 118	246 ± 114	503 ± 12.5	88.9 ± 8.36	358 ± 57.8	1420 ± 237 a	247± 152 a

All units in kg N ha^{-1} unless otherwise specified.
When compared within species, means followed by the same letter are not significantly different at $P > 0.05$ by Tukey's test.

the mineralization of organic N. Similarly, substantial leaching loss depleted the N applied in the fertilizer.

The NOx-N, NHx-N and the N capital did not differ significantly between treatment and the control. Leachate treatment did not lead to a significant increase in the N capital, but the components of the N capital changed in response to the large influx of NHx. The addition of NHx and nitrification in the soil led to a marked elevation in the levels of NHx-N and NOx-N. However, the TKN decreased slightly after leachate treatment and this may be attributed to the mineralization and leaching loss of organic N.

With vegetation, leachate irrigation in general led to elevation in the N capital. Both the N reserve in the soil and biomass increased after receiving leachate (Table 4.4). However, the change in the N capital after fertilizer application varied with the vegetation type. Gains in the N capital were observed only in the two grass species. The rapid uptake immobilized over 500 kg N ha^{-1} in the aboveground biomass of *Vetiveria zizanioides* and in the underground biomass of *Paspalum notatum*. Moreover, soil planted with grasses had higher TKN content compared with that planted with trees. The finer root system and higher transpiration rate of the two grasses reduced the leaching loss more effectively. Trees receiving fertilizer showed a decrease in the N capital, as the leaching loss was greater than the N accumulation in biomass. However, in the leachate treatment, trees performed better than grasses in rebuilding the N capital. The gain in N capital by *Hibiscus tiliaceus* and *Litsea glutinosa* were 1050 and 919 kg N ha^{-1}, respectively, nearly doubled that by the two grasses under leachate irrigation. Trees were benefited by a relatively high influx of N from leachate. High growth and uptake increased the N in biomass. The growth of grasses was also promoted by leachate, but to a lesser extent. For example, owing to the slow growth of *Paspalum notatum* in the leachate treatment, the change in the biomass N in the leachate treatment was much lower that in the fertilizer treatment. Moreover, the soil NHx-N and TKN was exceptionally low in the leachate treatment of vetiver grass, which may be attributed to the increased NH_3 volatilization. The massive root system, large surface area of grass leaves and the lower NH_3 compensation point of mesophyll cells facilitate the volatilization of NH_3 to air.

The results suggest the importance of vegetative cover to the development of the soil N capital. Leachate and fertilizer led to an immediate increase in the available N in soil. However, in the absence of vegetation, the applied N, as well as the existing N in the soil, was susceptible to leaching loss. Plant uptake and assimilation incorporated the available N into biomass and litter for long-term storage.

4.3.4.2 Leaching loss

Leaching loss was the only measurable means of nutrient loss in the current study. When compared within vegetation type, leaching loss was greater in the leachate treatment because of the high N influx (Table 4.5). Although leachate provided N mainly in the form of NHx, NO_3^-, a product from nitrification, was the major form of N loss in the leachate treatment. In columns without vegetation, NO_3^- leaching comprised of 78.3% and 94.4% of the N loss in the leachate and fertilizer treatments

Table 4.5 N loss during the leachate irrigation experiment.

Vegetation type	Treatment	Percolate			Leaching	Unaccountable
		NOx-N	NHx-N	TKN	loss	loss
Without vegetation	Water	9.63 ± 6.12	1.60 ± 0.15	2.04 ± 0.21	13.9 ± 9.19	598 ± 77.2 b
	Fertilizer	170 ± 27.4	2.31 ± 1.08	2.87 ± 1.40	180 ± 27.3	549 ± 131 b
	Leachate	199 ± 35.6	43.3 ± 32.1	54.5 ± 40.4	254 ± 62.2	1560 ± 96.8 a
Hibiscus tiliaceus	Water	11.7 ± 16.3	0.90 ± 0.61	1.14 ± 0.78	14.2 ± 20.3	852 ± 56.6 a
	Fertilizer	73.7 ± 26.6	2.3 ± 2.26	2.80 ± 2.74	79.4 ± 36.4	163 ± 23.9 b
	Leachate	121 ± 50.6	15.1 ± 19.3	19.1 ± 24.2	113 ± 85.2	759 ± 203 a
Litsea glutinosa	Water	6.48 ± 3.61	1.40 ± 0.35	1.74 ± 0.46	14.2 ± 3.58	901 ± 7.38 a
	Fertilizer	62.8 ± 28.3	1.34 ± 0.51	1.70 ± 0.67	51.7 ± 14.7	365 ± 27.6 b
	Leachate	87.5 ± 19.7	17.0 ± 16.6	21.0 ± 20.3	86.9 ± 32.7	913 ± 156 a
Paspalum notatum	Water	5.39 ± 2.67	1.89 ± 0.81	2.29 ± 0.85	7.95 ± 2.83	625 ± 64.6 b
	Fertilizer	84.6 ± 10.6	1.31 ± 0.37	1.66 ± 0.44	80.4 ± 2.68	47.4 ± 43.8 c
	Leachate	225 ± 50.3	32.2 ± 16.6	39.9 ± 20.1	299 ± 4.34	1350 ± 173 a
Vetiveria zizanioides	Water	2.95 ± 1.05	0.89 ± 0.15	1.12 ± 0.16	4.91 ± 1.18	841 ± 75.6 b
	Fertilizer	4.62 ± 4.90	0.43 ± 0.20	0.45 ± 0.42	5.07 ± 5.30	-330 ± 285 c
	Leachate	90.4 ± 21.4	5.97 ± 5.75	7.68 ± 7.41	96.0 ± 29.3	1700 ± 213 a

When compared within species, means followed by the same letter are not significantly different at $P > 0.05$ by Tukey's test.
All units in kg N ha^{-1} unless otherwise specified.

respectively. Retention by CEC, fixation by clay and nitrification reduced the amount of leaching loss in the form of NHx.

The increased nutrient uptake (up to 1000 kg N ha^{-1} in biomass) and evapotranspiration by the vegetative cover reduced the leaching loss of nitrogen. When compared with columns without vegetation, nutrient uptake and evapotranspiration of the vegetative cover at least halved the leaching loss of N in the leachate treatment, with the exception in columns planted with *Paspalum notatum*. *Vetiveria zizanioides* reduced the leaching loss in the fertilizer treatment by 97.0%. Soil planted with *Vetiveria zizanioides* only had leaching loss of 96.0 kg N ha^{-1}, which was the lowest among the four species.

In ecosystems where N is limiting, there is an intense competition for mineralized N, or the intermediates of the mineralization processes. Forest litter and soil typically interact with vegetation to form a closed cycle of N. Interactions among soil, plants and soil microbes contribute to about 95% of the global flow of N (Stevenson and Cole, 1999). The rate of N uptake is limited by and approximately balances the annual N return to the forest floor (Tamm, 1991). Small amount of soil NHx is left for microbial nitrification and leaching loss. High levels of NO_3^- leaching indicated N saturation, a condition where the N availability exceeds the nutritional demands of plants and soil microorganisms (Ågren and Bosatta, 1988; Aber *et al.*, 1989).

4.3.4.3 Unaccountable N loss

Unaccountable N loss (N_{unacc}) (Table 4.5) refers to the loss of N from the soil-plant system which could not be identified and quantified by the present experimental design. It is calculated based on the sum of the initial N capital and the external input, minus the loss in percolate and the final N reserve in soil and plants. It can be attributed to NH_3 volatilization, dentrification and the N loss in litter. Discussion will focus on the gaseous loss of NH_3, since the N loss in denitrification and litter loss were less important compared with NH_3 volatilization.

NH_3 volatilization

At one time, it was thought that the presence of NH_3 in the atmosphere was of little importance, because of its low concentration. However, in the past two decades, it has been realized that there was a cycling of significant amounts of NHx through the plants, soil and atmosphere. Considerable attention has been given in recent years to the loss of NH_3 by application of NH_4^+-containing fertilizers (such as urea, anhydrous NH_3 and farmyard manure to soils).

There is limited information about NH_3 volatilization from leachate-irrigated lands. In the present study, it comprised 19 - 67% of leachate N applied. Over 80% of the N was applied in NHx form, which was susceptible to volatilization. The unaccountable N loss in the leachate treatment was nearly double that in the fertilizer treatment.

Ammonium ions (NH_4^+) in the soil solution form an equilibrium with ammonia (NH_3). NH_3 is subject to gaseous loss to the atmosphere. Volatilization of NH_3 could commence immediately after fertilizer application and proceed at a high rate. Depending on soil moisture content, 20 to 50% of injected NH_3 can be lost within a few hours (Sommer and Christensen, 1991). Similar results were observed on pasture with manure application. NH_3 volatilization as high as 12 kg N ha^{-1} h^{-1} was recorded immediately after slurry application (Pain *et al.*, 1989). Half of the NH_3 loss occurred within 4 to 12 h (Pain *et al.*, 1989; Moal *et al.*, 1995).

The quantity of NH_3 volatilization depends on the type and timing of fertilizer application, soil type and the environmental conditions at the time of application. Soil factors which speed up the volatilization include low cation exchange capacity (CEC), high soil pH and low moisture content. NH_4^+ reacts readily with the cation exchange complex in soil and thus reduces the amount of NHx in soil pore water. Elevated soil pH increases the dissociation of NH_4^+ to NH_3. The fraction of NH_3 in soil solution increases by an order of magnitude for every pH unit above 6.0 and thus increases the amount of NH_3 volatilization from soil (Cai, 1997). Climatic factors include air (soil) temperature and wind speed. Higher temperature reduces the solubility of NH_3 in water and increases the NH_3:NH_4^+ at a given soil pH. High wind speed promotes the rapid transport of NH_3 away from the surface (Cai, 1997). Management practices that decrease the soil pH, increase CEC (e.g. by adding zeolite), or move the fertilizer deeper into the soil profile (e.g. by subsurface irrigation) can reduce NH_3 loss. Also, NH_3 volatilization would be lower when fertilizer is applied in cooler and less windy days.

Vegetative cover plays an important role in regulating the NH_3 volatilization. It is generally believed that NH_3 volatilization is reduced in the presence of growing plants. Not only was the soil NH_4^+ level reduced through plant uptake, but some of the evolved NH_3 may be reabsorbed by the plant canopy. In the fertilizer treatment of this study, columns with plants had a lower N_{unacc} than those without vegetation. *Hibiscus tiliaceus* and *Litsea glutinosa* with leachate application also had a lower N_{unacc} than the leachate treatment without vegetation. Negative N_{unacc} was observed in the vetiver grass and *Paspalum notatum* treated with fertilizer, which implied a net gain in N. The reason for negative N_{unacc} was unclear, possibly because of the uptake of gaseous NH_3 emitted from the nearby columns.

Plants can serve both as a sink and as a source of atmospheric NH_3. Whether NH_3 is emitted or adsorbed by a plant depends upon the NH_3 compensation point of the plant (i.e., the concentration of atmosphere NH_3 concentration at which no net exchange of NH_3 occurs between the liquid and gaseous phases). Net output of NH_3 is observed when the atmospheric NH_3 concentration is lower than the compensation point of the leaf mesophyll cell (Farquhar *et al.*, 1980). The net NH_3 exchange is likely dependent on several interacting factors like plant type, temperature, phenological growth stage and time of a day (Farquhar *et al.*, 1980; Harper *et al.*, 1987; Harper and Sharpe, 1995). The amount of NH_3 loss, for example in corn, can vary from 1 - 2 kg N ha^{-1} (Schjorring, 1995) to 15 kg N ha^{-1} (Harper *et al.*, 1987).

It has been suggested that soil N supply affects the NH_3 compensation point. Harper *et al.* (1987) suggested a direct relationship between plant N status, fertilizer application and NH_3 loss. In another study oilseed rape exposed to 0.5 mol N showed a compensation point about 10 times higher than plants supplied with 0.15 mol N (Husted and Schjoerring, 1996). Such results may be explained by the NH_4^+ uptake via xylem which leads to elevated concentrations in the apoplastic space and therefore an increased compensation point. In the present study, plant uptake reduced the amount of soil NHx and thus lowered the rate of NH_3 volatilization under moderate application of N. However, when the influx of NHx was markedly increased in the leachate treatment, increase in the compensation point would lead to foliar emission of NHx and resulted in a larger N_{unacc} in the leachate treatments.

Moreover, when comparing the leachate treatments among different species, the N_{unacc} of trees was only a half of that of the grasses which can be attributed to their different NH_3 compensation points. Field studies have shown that herbaceous plants have a higher compensation points than trees (Geßler and Rennenberg, 1998). NHx in trees was less susceptible to volatilization under a given set of atmospheric conditions.

When NH_3 is evolved from the soil surface, it either redeposits near the source of emission or dissolves in moisture and reacts readily with acidic gases, forming aerosols of $(NH_4)_2SO_4$ or NH_4NO_3. NH_3 can travel a long distance in the atmosphere. Modelling estimates indicate that about 50% of emitted NH_3 is redesposited on the earth surface within 50 km from the emission source. However, if gaseous NH_3 reaches the mixing layer of the troposphere, the half-life can reach 3 to 6 hours over a distance of 65 to 130 km (Ferm, 1998).

Substantial amounts of NHx-N may be deposited in the nearby ecosystem. Hutchison and Vitets (1969) demonstrated that a 90000 head cattle feedlot provided N deposition of about 50 kg N^{-1} ha^{-1} y^{-1}, nearly a quarter of the annual N requirement (Bradshaw, 1983), within few km from the feedlot.

Denitrification

While the autotrophic nitrifiers use energy by converting NH_4^+ to a more oxidized form, denitrifying bacteria exploit the oxygen in NO_3^- or NO_2^- as electron acceptor when oxygen supply is scarce.

	NO_3^-	$\rightarrow$	NO_2^-	$\rightarrow$	NO	$\rightarrow$	N_2O	$\rightarrow$	N_2
	nitrate		nitrite		nitric oxide		nitrous oxide		nitrogen
Oxidation state	+5		+3		+2		+1		0

Denitrification can also occur as a purely chemical process. Nitrite can react with NHx, Fe(II) or amine groups to yield molecular N. Higher fertility forests have a high turnover of C and N to microbes, including higher denitrification rates. The N_2O fluxes in humid tropical forest soils of different fertility were 0.47 - 2.6 kg N ha^{-1} y^{-1} (Matson and Vitousek, 1990).

Nearly all denitrifying bacteria are strict anaerobes. Oxygen acts as an inhibitory factor of denitrification by interfering with the synthesis and activity of the enzymes. NO_3^- reduction was undetected when soil electrical potential (E_h) was above 400 mV (Asghar and Kanehiro, 1976). Nitric oxide reductase and nitrous oxide reductase are more sensitive to oxygen than other enzymes. The critical E_h for the reduction of N_2O to N_2 appears to be 200 - 240 mV (Focht and Verstraete, 1977). Erich *et al.* (1984), showed that exposing soil to air for 24 hours did not influence the formation of N_2O, but completely inhibited its reduction to N_2 in soil. In the present study, the E_h of soil of different depths ranged between +261 and +359 mV. Significant differences between vegetation types and treatments were not observed. The good aeration of the loamy sand soil was not favourable to denitrification. The amount N loss by denitrification was negligible compared with NH_3 volatilization and leaching.

Loss with litter

A small fraction of N_{unacc} can be attributed to litter fall to soil, which was not collected and measured in this study. During the course of the experiment, plants grew rapidly under warm greenhouse condition and adequate water supply. Most of the plants did not senescent as the foliage tissue was still young. Only small amount of litter were produced.

Even if organic matter returns to the forest floor, its contribution to the N turnover in the experimental period was small. Only a small fraction of the total N in soils, generally below 0.1%, exists in plant as available mineral compounds at any one time (as NO_3^- or exchangeable NHx) (Stevenson, 1982). Thus only a few kg N ha^{-1} may be immediately available to the plant, even though as much as thousands of kg N ha^{-1} may be present in litter fall. Moreover, degradation products like amino acids and nucleic acids are attractive substrates for microorganisms, which often can be used as a carbon or N sources. Most of the N is taken up by the microorganisms themselves. The contribution of litter to the N balance was of the least importance.

4.4 Conclusions

This study evaluated the efficiency of the use of leachate N by the soil-plant system. The absence of growth inhibition in the recipient plants again proved that germination tests using *Brassica chinensis* and *Lolium perenne* were sensitive to the phytotoxicity of landfill leachate and the EC50s determined can be a safe upper limit of the leachate application rate. The growth performance in the leachate and fertilizer treatments did not differ significantly in most plant species tested, indicating that both the leachate and mineral fertilizer were effective sources of plant nutrients. However, suboptimum growth and stress symptoms in *Paspalum notatum* to leachate exposure suggested that leachate application may not be suitable for young grass seedlings when the contact between leachate with foliage cannot be avoided. Also germination test using the seeds of recipient plants species may better reflect their responses in field conditions.

However, it seems that the application rate was too high at the early stage of vegetation establishment. The plant uptake could not keep pace with the excessive supply of N from leachate. Saturation of NHx in soil, together with the subsequent nitrification, led to massive N loss in the form of NO_3^- leaching. With consideration to the actual nutrient requirement of plants, there was an opportunity for reducing the application rate to alleviate leaching loss.

Vegetation played an important role in the development of N capital. Soil with vegetative cover in general had lower leaching loss and higher accumulation in soil N capital. Evapotranspiration and plant uptake reduced the soil moisture content and percolate volume. Moreover, higher tissue N content and better vegetative growth could incorporate the applied N to the biomass, resulting in an increase in the N capital of the soil-plant system.

Chapter 5 General conclusion

Irrigation with landfill leachate provides a means of wastewater disposal as well as nutrient reuse. Leachate contains considerable amounts of NHx and other nutrients which can be assimilated for plant growth. This project aimed at evaluating the feasibility of using landfill leachate as an alternative N source to fertilizer, and making use of phytotoxicity data in designing leachate irrigation plans to protect the plants from growth retardation or death.

5.1 Summary of findings

Leachate samples from 5 local landfills of different ages were assayed for their chemical properties and phytotoxicity (Chapter 2). Both were characterized by high levels of salts, NHx and high TOC, but low levels of heavy metals. This provided an opportunity of using landfill leachate for irrigation purposes.

Seed germination/root elongation tests using the seeds of *Brassica chinensis* and *Lolium perenne* were conducted to evaluate the phytotoxicity of leachates. The seeds showed a dichotomous response to some leachate samples. Besides the effects of the nutritive constituents, the concept of hormesis was introduced to explain the growth stimulation at low dose (concentration). The EC50 ranged between 4 - 30% v/v and in general decreased with the strength of leachate samples.

The second experiment (Chapter 3) aimed at incorporating the phytotoxicity information from germination tests into a leachate irrigation trial. Seedlings of 12 tree species were tested under irrigation of leachates at their respective EC50 levels. No tree mortality or growth inhibition was observed in 90 days of leachate application. Chlorophyll fluorescence measurements also showed that plants receiving leachate did not suffer from a reduction in the photosynthetic efficiency. Leachate enhanced the growth of tree seedlings. *Litsea glutinosa* and *Hibiscus tiliaceus* had remarkable growth, and the other non N-fixers were not inferior to the N-fixing *Acacia auriculiformis*. The extent of growth stimulation induced by two leachates did not differ significantly in most of the species, indicating that with proper dilution, both the leachates from young and old landfills were suitable for irrigation purposes. The seed germination tests provided a conservative estimate of the phytotoxicity of landfill leachate. Irrigated plants can be protected from growth inhibition when leachate irrigation plans are designed with reference to phytotoxicity data.

Application of leachate improved the soil N content, though phosphorus deficiency is a problem. Nitrification of NHx in leachate resulted in a marked increase in the soil NO_3^- content. Although soil salinity also increased considerably after leachate irrigation, the resulting soil EC was far below the threshold of saline soil. It should be noted that the potential hazard of salts may be exacerbated when salts are left behind by evapotranspiration. In addition to the dilution level, adequate water should be applied to elute the salts from the rooting zone.

The irrigation experiment has shown that phytotoxicity tests using germinating seeds provided a safe upper limit of the application rate. However, whether the application rate is optimal for revegetation purposes depends on the ability of vegetation to take up and utilize the applied nutrients for growth. Understanding the fate and behaviour of applied N can help to improve leachate irrigation practice and to mitigate the environmental impacts of excess N application. The final experiment (Chapter 4) adopted a mass balance approach to investigate the distribution of leachate N in plants, soil and soil percolate. The soil-plant system that received leachate at the EC50 level was compared to treatment with mineral fertilizer (Nitrophoska 15:15: 15). In addition to the 2 tree species selected based on the results of the previous study (Chapter 3), 2 grasses were included for comparison. Their growth performance again confirmed that leachate applied at the EC50 level could ensure that the plants would not show growth inhibition. Plant growth in the leachate and fertilizer treatments did not differ significantly; three species out of the 4 were benefited by leachate application.

The results of the N balance study also suggested the role of plants in retaining nutrients. In treatment groups without vegetative cover, a substantial amount of N was lost in soil percolate; 90% of N applied was lost in leaching. Plant limited the amounts of ions in soil pore water and their movement by evapotranspiration and uptake. In some cases, vegetative cover reduced the leaching loss by 50%.

In the absence of plants, the soil N capital did not increase after leachate application. Even though the soil contents of NHx and NO_3^- increased substantially, N added with leachate was too low to compensate for the leaching loss. In contrast, with vegetative cover, a 12-week treatment with leachate brought about 250 - 1050 kg N ha^{-1} accumulated in biomass and soil. *Vetiveria zizanioides* exhibited a good performance in limiting the volume of soil percolate as well as the N loss with it. Vetiver grass can be planted to control the hydraulic loading of landfill soil cover and prevent percolate from moving out of leachate-irrigated sites. However, the gain in N capital was not found in the treatment groups of vetiver grass, possibly because of enhanced N loss via NH_3 volatilization.

Both the leachate irrigation experiment (Chapter 3) and N balance study (Chapter 4) showed that substantial amounts of NO_3^- were liberated in the nitrification of the NHx in leachate. The high leaching loss in the N balance study suggested that the application rates being tested exceeded the nutrient (especially N) needs of plants. Leaching of excess N not only causes water pollution, but may also interfere with the natural succession process.

5.2 Ecological consequence of increased and excess N deposition

Figure 5.1 illustrates the changes of plants and soil conditions to chronically elevated N deposition. Terrestrial ecosystems are often N-limited, either because of low total reserve or because of low availability (Stage 0). Forest litter and soil typically interact with vegetation to form a closed cycle of N in which the annual rate of N uptake per unit area is limited by and approximately balances the annual N return to the forest floor (Tamm, 1991). About 95% of the N that cycles annually within the pedosphere interacts solely within the soil, plant and soil microbes (Stevenson and Cole, 1999). This closed cycle differs from the open cycle of wetlands and fertilized croplands where primary production is mainly supported by external N supply.

The addition of N, either by fertilizer application or increased atmospheric deposition, is then likely to increase the growth of some organisms (Stage I). The first consequence of elevated N supply to an N-limited forest will most probably be increased vegetative growth and the accumulation of N in both biomass and soil. Luxury consumption occurs when N is taken up in excess of the plant's physiological demands (Dueck and Van der Eerden, 2000). As demonstrated in the previous experiment (Chapter 4), foliar biomass and N content increased with N supply. Even with an unchanged C:N ratio, a larger amount of litter would mean greater N turnover in a given time.

Increased N deposition in forests has the potential to shift the closed N cycle to a relatively open status by increasing the importance of N inputs and outputs relative to the rate of internal cycle (Tamm, 1991). However, the increase in vegetative growth may soon be suppressed by increased shading from a denser canopy. Fast-growing N-demanding species may have a competitive advantage over the N-tolerant ones. Legumes and other symbiotic N-fixers would stop fixation and thereby lose their competitive advantage. Leachate application may result in similar consequences as different growth stimulation and inhibition to nodule formation in the N-fixing species were observed in the previous experiment (Chapter 3).

Therefore, an increased N deposition means a change in the competitive advantages among species. The vegetative component is expected to change to less symbiotic N-fixers, less N-tolerant species and more N-demanding plants. Similarly, the community composition of 'non-tree' ecosystems such as grasslands are expected to

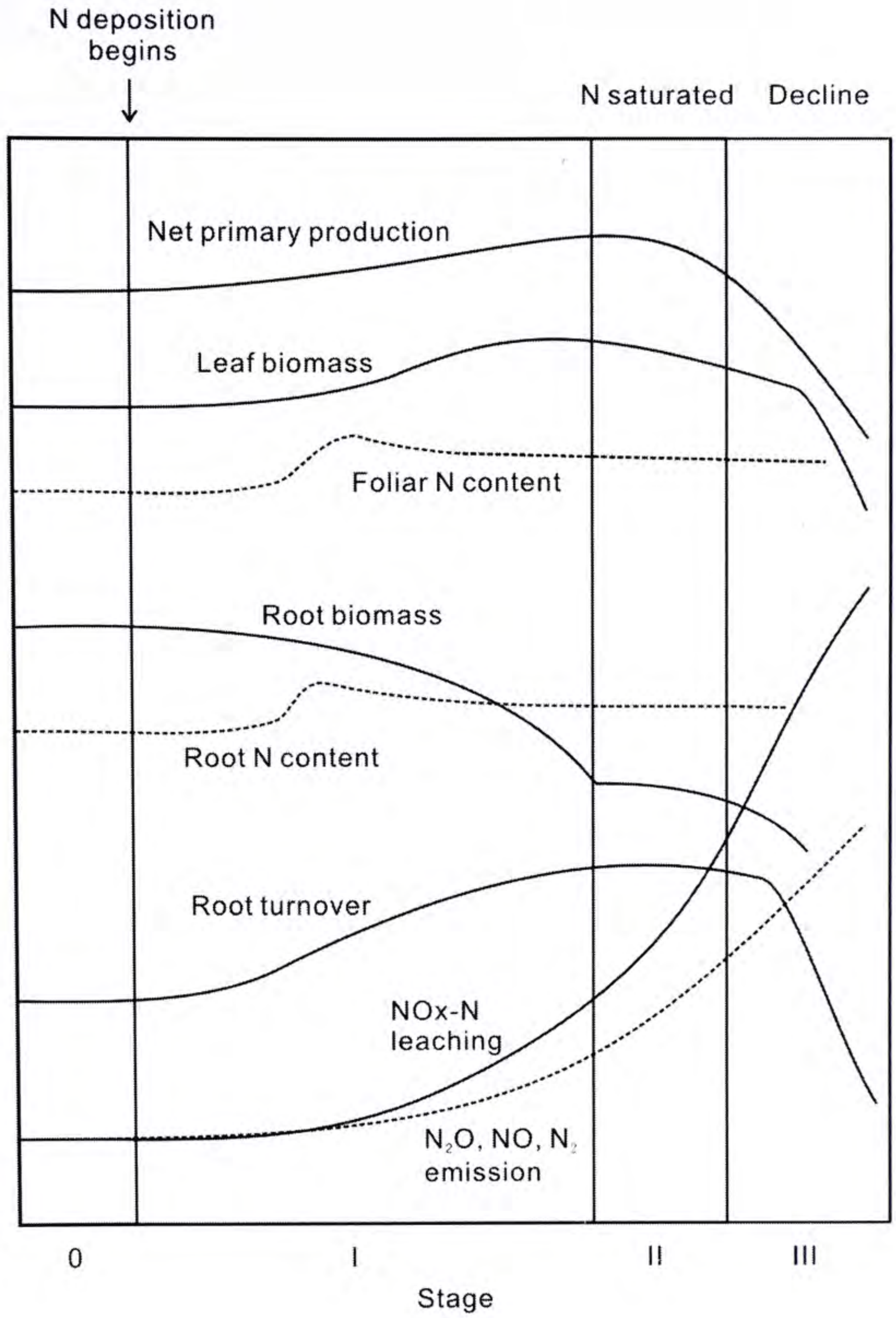


Figure 5.1 Conceptual model showing forest responses to chronically elevated N deposition. Stage 0 represents the pristine condition. Stage I follows the onset of elevated N deposition. When N is still limiting, addition of N leads to elevated net primary production. Increases in the biomass and tissue N contents leads to higher N turnover rate. NO_3^- loss above the baseline level may occur. If forests proceed to Stage II, increase in NO_3^- loss and biomass are more evident. Further increase in N deposition may result in forest decline (Stage III). Tree mortality and reduction in productivity occur. The variations of lines along the y-axis indicate the qualitative change of the individual processes or properties, which are not drawn to scale (adapted from Aber *et al.*,1989, 1998; Nadelhoffer, 2000).

change considerably with increasing N level. Those plants with large height increments will be more competitive. A few tall grasses together with some tall shrubs are expected to become dominant and allow few other species to coexist.

N saturation (Stage III) occurs when the primary production is not increase further by increased N supply. Nitrification of the surplus N and subsequent leaching may lead to soil acidification. The loss of N may exceed the input over a rather long period of time. Moreover, excessive foliar-N may disturb the nutrient balance and increase the sensitivity to frost, drought, pests and pathogens (Van Dijk and Roelefs, 1988; Dueck *et al.*, 1991). Generally young, rapid-growing and well-nourished plants are more likely to suffer from attack by pests. A high content of amino acids in the plants may result in a more severe attack by sucking parasites (Marschner, 1995). Change in the vulnerability of plants, together with the soil acidification, may finally lead to the decline of forest.

5.3 Research prospects

The present study suggested the beneficial effect of landfill leachate for irrigation purposes when it is properly diluted in light of phytotoxicity information. However, uncertainty of the ecological consequences of leachate irrigation still exists. In future, a pilot-scale field study should be undertaken to investigate the long term effects of leachate irrigation on plants and soil.

Research using farm yard manure and mineral fertilizers has shown that the addition of N in different amounts led to entirely different results. The increased N supply from leachate may lead to similar consequences. In addition, relatively high salt content in leachate may affect the natural succession process on leachate-irrigated sites. It is anticipated that N-demanding species with higher salt tolerance may have competitive advantage. Therefore, besides the growth performance of the recipient plants, changes in the community composition, including the effects on soil fauna, can be addressed in further studies.

Nitrification activity in soil was enhanced after land application of leachate. Other microbial processes which may be critical to nutrient cycling in soil may also be affected. Leachate application may affect mineralization in two ways. C and N sources in leachate may enhance the growth of the microbial community in soil, while soil acidification and the potent constituents in leachate may inhibit their activity. Reduced C:N ratio in litter may be less favorable to mineralization. Moreover, it is anticipated that under ample supply of N, the importance of N fixation to the ecosystem will decrease, including (but not limited to) symbiotic N fixation and that accomplished by cyanobacteria. The rates of nitrification and denitrification in soil will increase in response to elevated N turnover. A better understanding about the changes in these processes will help to improve the management practices on leachate-irrigated areas.

Research on N-enriched croplands and forests was demonstrated the importance of NH_3 volatilization to the N budget. NH_3 volatilization would inevitably have a great influence on the N balance of leachate-irrigated lands and the surrounding areas, since the N is supplied mainly in the form of NHx. However, there is little information about the N loss from leachate-irrigated soils. Micrometeorological methods have been recommended for the estimation of the NHx loss from croplands, over a relatively large area with radius of 100 meters. Two techniques, gradient diffusion and mass balance methods (Denmead, 1983), have been used for assessing NH_3 volatilization without disturbing the environmental conditions. To further improve the accuracy of measurements, spiking the leachate with ^{15}N-labelled $(^{15}NH_4)_2SO_4$ to trace the fate of the applied NH_4^+ in field situation, including NH_3-volatilization should be considered. Estimating NH_3 loss can provide information for calibrating the N application rate by making allowance for N loss in NH_3 volatilization.

Landfill leachate irrigation does have its drawbacks and problems, yet it represents unique opportunities. Leachate contains considerable amounts of NHx and other constituents that can serve as an alternative source of plant nutrients. However, the methods of application should be carefully designed, with the aid of phytotoxicity tests to evaluate the toxicity of different leachates. Also the assimilation capacity of soil-plant systems and the N requirement in different stages of succession should also be considered so as to meet the N demand for plant growth and protect ecosystems from N saturation.

References

Aber JD, McDowell W, Nadelhoffer K, Magill A, Bertson G, Kamakea M, McNulty S, Currie W, Rustad L and Fernandez I 1998. Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. *Bioscience* 48:921-934.

Aber JD, Nadelhoffer KJ, Streudler P and Melillo JM 1989. Nitrogen saturation in northern forest ecosystem. *Bioscience* 39:378-386.

Ågren GI and Bosatta E 1988. Nitrogen saturation in terrestrial ecosystems. *Environmental Pollution* 54:185-197.

Allen SE. 1989. *Chemical Analysis of Ecological Materials*, 2nd edition. Blackwell Scientific Publications, Boston.

American Public Health Association 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th edition. American Public Health Association, New York.

Andreottola G and Cannas P 1992. Chemical and biological characteristics of landfill leachate. *In:* Christensen TH, Cossu R and Stegmann R, editors. *Landfilling of Waste: Leachate,* pp 65-88. Elsevier Science Publishers Ltd., Essex.

Armentano TV and Bennett J 1992. Air pollution effects on the diversity and structure of communities. *In:* Barker JR and Tingey TD, editors. *Air Pollution Effects on Biodiversity,* pp 159-176. Van Nostrand Reinhold, New York.

Asghar M and Kanehiro Y 1976. Effects of sugarcane trash and pineapple incorporation on soil nitrogen, pH and redox potential. *Plant and Soil* 44:209-218.

Ayers RS and Westcot DW 1985. Water quality for agriculture, FAO irrigation and drainage paper 29. Food and Agriculture Organization of the United Nations.

Bagchi A 1987. Natural attenuation mechanisms of landfill leachate and effects of various factors on the mechanisms. *Waste Management and Research* 5:453-464.

Barlaz MA, Ham RK and Schaefer DM 1989. Mass balance analysis of anaerobically decomposed refuse. *Journal of Environmental Engineering* 115:1088-1102.

Barraclough D 1995. ^{15}N isotope dilution techniques to study soil nitrogen transformations and plant uptake. *Fertilizer Research* 42:185-192.

Baum B and Parker CH 1974. *Solid Waste Disposal.* Ann Arbor Science, Ann Arbor.

Bell RW 1999. Diagnosis and prognosis of soil fertility constraints for land restoration. *In:* Wong MH, Wong JWC and Baker AJM, editors. Remediation and Management of Degraded Lands, pp 163-174. Lewis Publishers, Boca Raton.

Bewley JD and Black M 1994. *Seeds: Physiology of Development and Germination*, 2nd edition. Plenum Press, New York.

Bowman MS, Clune TS and Sutton BG 2002. Sustainable management of landfill leachate by irrigation. *Water, Air, and Soil Pollution* 134:81-96.

Boyle WC and Ham RK 1974. Biological Treatability of landfill leachate. *Journal Water Pollution Control Federation* 46:860-872.

Bradshaw AD 1983. The reconstruction of ecosystems. *Journal of Applied Ecology* 20:1-27.

Bradshaw AD 2002. The development of nitrogen capital. *In:* Wong MH and Bradshaw

AD, editors. *The Restoration and Management of Derelict Land: Modern Approaches*, pp 128-137. World Scientific, River Edge.

Brady NC 1990. *The Nature and Properties of Soil*, 10th edition.: Macmillan Publishing Company, New York.

Brain P and Cousens R 1989. An equation to describe dose responses where there is stimulation of growth at low dose. *Weed Research* 29:93-96.

Bramyrd T 1988. Leachate from landfills – a valuable fertilizer for revegetation of landfills with grass or energy forests. *In:* Andersen L and Moller J, editors. *ISWA 88*, proceedings of the 5th International Solid Wastes Conference, International Solid Wastes and Public Cleansing Association; 11-16 Sep 1998; Copenhagen, Denmark. pp 61-68. Academic Press, London.

Budavari S, Smith A, Heckelman PE and Kinneary JF 1996. The Merck Index, 12th edition. Merck and Co. Inc., Whitehouse Station.

Cai GX 1997. Ammonia volatilization. *In:* Zhu ZL, Wen QZ and Freney JR, editors. *Nitrogen in Soils of China,* pp 192-214. Kluwer Academic Publishers, Dordrecht.

Cai GX, Zhu ZL, Zhu ZW, Trevitt ACF, Freney JR and Simpson JR 1985. Nitrogen loss from ammonium bicarbonate and urea applied in flooded rice field. *Soils* (in Chinese) 17:255-229.

Calabrese EJ and Baldwin LA 1997. The dose determines the stimulation (and poison): development of a chemical hormesis database. *International Journal of Toxicology* 16: 545-559.

Calabrese EJ and Baldwin LA 1998. Hormesis as a biological hypothesis. *Environmental Health Perspectives Supplements* 106:357-362.

Calabrese EJ and Baldwin LA 2000. Chemical hormesis: its historical foundations as a biological hypothesis. *Human & Experimental Toxicology* 19:2-31.

Carra JS and Cossu R 1990. *International Perspectives on Municipal Solid Wastes and Sanitary Landfilling.* Academic Press Ltd., London.

Carville MS and Robinson HD 1991. Landfill leachate in Hong Kong characterization and treatment with special reference to ammonia removal. *In:* Boxall J, editor. *Proceedings of Polmet '91: Pollution in The Metropolitan and Urban Environment*, Hong Kong Institution of Engineers; 9-13 December 1991; Hong Kong. pp 141-167.

Census and Statistics Department (Hong Kong) (CSD) 2003. Hong Kong in figures. Retrieved 23 Sep 2003, from Census and Statistics Department website: *http://www.info.gov.hk/censtatd/eng/hkstat/index2.html*

Chan KY 1982. Change to a soil on irrigation with a sanitary landfill leachate. *Water, Air, and Soil Pollution* 17:259-304.

Chan KY, Davey BG and Geering HR 1978. Interaction of treated sanitary landfill leachate with soil. *Journal of Environmental Quality* 7:306-310.

Chan YSG, Wong MH and Whitton BA 1999. Effects of landfill leachate on growth and nitrogen fixation of two leguminous trees (*Acacia confusa* and *Leucaena leucocephala*). *Water, Air, and Soil Pollution* 111:29-40.

Chen T, Esnault D and Koenig A 1997. First year operation of the NENT landfill leachate treatment works in Hong Kong. *In:* Margherita di Puka S, editor. *Proceedings Sardinia 1997, Sixth International Landfill Symposium*; 13-17 Oct 1997; Cagliari, Italy.

CISA Environmental Sanitary Engineering Centre, Cagliari.

Chong SL 1999. Restoration of degraded lands in Hong Kong. *In:* Wong MH, Wong JWC and Baker AJM, editors. *Remediation and Management of Degraded Lands,* pp 305-317. Lewis Publishers, Boca Raton.

Chu LM, Cheung KC and Wong MH 1994. Variation in the chemical properties of landfill leachate. *Environmental Management* 18:105-117.

Clements FE, Goldsmite GW 1924. *The Phytometer Method in Ecology: The Plant Community as Instruments.* Garnegie Institution of Washington, Washington.

Corlett RT 1999. Environmental forestry in Hong Kong: 1987-1997. *Forest Ecology and Management* 116:93-105.

Cossu R 1989. Role of landfilling in solid waste management. *In:* Christnensen TH, Cossu R and Stegmann R, editors. *Sanitary Landfilling: Process, Technology and Environmental Impact*, pp 3-9. Academic Press, Washington.

Cramer GR, Lauchli A and Polito VS 1985. Displacement of Ca^{2+} by Na^{+} from the plasmalemma of roots. *Plant Physiology* 79:207-211.

Cureton PM, Groenevelt PH and McBride RA 1991. Landfill leachate recirculation: effects on vegetation vigor and clay surface cover infiltration. *Journal of Environmental Quality* 20:17-24.

Davies RS, Younger A, Hodgkinson R and Chapman R 1998. Nitrogen loss from a soil restored after surface mining. *In:* Fox HR, Moore HM and Mackintosh AD, editors. *Land Reclamation: Achieving Sustainable Benefits,* pp 235-240. Balkema, Rotterdam.

Denmead OT. 1983. Micrometeorological methods for measuring gaseous losses of nitrogen in the field. *In:* Freney JR and Simpson JR, editors. *Gaseous Loss of Nitrogen from Plant-Soil Systems,* pp 133-157. The Hague, Boston.

Department of Justice (Hong Kong) 1997. Technical memorandum Standards for effluents discharged into drainage and sewerage systems, inland and coastal waters (Laws of Hong Kong CAP 358AK). Retrieved 16 Sep 2003, from the BLIS database website: *http://www.justice.gov.hk/eng/index.htm*

Department of the Environment (United Kingdom) 1986. Waste management paper No. 26: landfill wastes. HMSO, London.

Devare M, Bahadir M 1994. Biological monitoring of landfill leachate using plants and luminescent bacteria. *Chemosphere* 28:261-271.

Dobermann A, Goovaerts P and George T 1995. Sources of soil variation in an acid ultisol of the Philippines. *Geoderma* 68:173-191.

Dreyfus BL, Diem HG, Freire J, Keya SO and Dommergues YR 1987. Nitrogen fixation in tropical agriculture and forestry. *In:* DaSilva EJ, editor. *Microbial Technology in the Developing World*, pp 7-50. Oxford University Press, Oxford.

Dueck Th A, Forel FG, Terhorst R and Van der Eerden LJ 1991. Effects of ammonia, ammonium sulphate and sulphur dioxide on the forest sensitivity of Scots pine (*Pinus sylvestris*). *Water, Air and Soil Pollution* 54:35-49.

Dueck Th A and Van der Eerden LJ 2000. Interactions between climate change and nitrogen deposition, with emphasis on ammonia. *In:* Singn SN, editor. *Trace Gas Emission and Plants*, pp 291-307. Kluwer Academic, Dordrecht.

Environmental Protection Department (Hong Kong) (EPD) 2001. Waste generation rates and forecast. Retrieved 30 Nov 2001, from Environmental Protection Department website: *http://www.info.gov.hk/wfbu/uowdn/wctb4.htm*

Environmental Protection Department (Hong Kong) (EPD) 2002. Information paper on future landfill development in Hong Kong. Retrieved 13 Sep 2003, from Environmental Protection Department website: *http://www.epd.gov.hk/epd/english/environmentinhk/waste/pub_consult/landfill_backgr_r01.html*

Environmental Protection Department (Hong Kong) (EPD) 2003a. *Environment Hong Kong 2003*. Government Printer: Hong Kong.

Environmental Protection Department (Hong Kong) (EPD) 2003b. Hong Kong's environment - waste. Retrieved 13 Sep 2003, from Environmental Protection Department website: *http://www.epd.gov.hk/epd/english/environmentinhk/waste/waste_maincontent.html*

Epron D, Dreyer E and Bréda N 1992. Photosynthesis of oak trees (*Quercus petraea* (Matt) Liebl.) during drought stress under field conditions: diurnal course of net CO_2 assimilation and photochemical efficiency of photosystem II. *Plant, Cell, and Environment* 15:809-820.

Erich MS, Bekerie A and Duxbury JM 1984. Activities of denitrifying enzyme in freshly sampled soils. *Soil Science* 138:25-32.

Ettala M 1987. Influence of irrigation with leachate on biomass production and evapotranspiration on sanitary landfill. *Aqua Fennica* 17:69-86.

European Communities 2002. Waste online material. Retrieved 13 Sep 2003, from Eurostat Website: *http://europa.eu.int/comm/eurostat/Public/datashop/print-product/EN?catalogue=Eurostat&product=env-pb-ws-EN&mode=download*

Farquhar GD, Firth PM, Wetselaar R and Weir B 1980. On the gaseous exchange of ammonia between leaves and the environment: determination of the ammonia compensation point. *Plant Physiology* 66:710-714.

Feigin A, Ravina I and Shalhevet J 1991. *Irrigation with Treated Sewage Effluent: Management for Environmental Protection*. Springer-Verlag, Hong Kong.

Ferm M 1998. Atmospheric ammonia and ammonium transport in Europe and critical loads – a review. *Nutrient Cycling in Agroecosystems* 51:5-17.

Fletcher JS, Johnson FL and McFarlan JC 1988. Data base assessment of phytotoxicity data published on terrestrial vascular plants. *Environmental Toxicology and Chemistry* 7:615-622.

Focht DD and Verstraete W 1977. Biochemical ecology of nitrification and denitrification. *Advances in Microbial Ecology* 1:135-214.

Frimmel FH and Weis M 1991. Aging effects of high molecular weight organic acids which can be isolated from landfill leachates. *Water Science and Technology* 23:419-426.

Fu LH 2004. The use of landfill leachate for growing ornamental plants [Final year project dissertation]. The Chinese University of Hong Kong, Hong Kong.

Fuller WH and Warrick AW 1986. *Soils in Waste Treatment and Utilization*, Vol. 2. CRC Press, Boca Raton.

Garcia C and Hernández T 1996. Influence of salinity on the biological and biochemical activity of a calciothird soil. *Plant and Soil* 178:255-263.

Gasser JKR 1973. An assessment of the importance of some factors causing lime loss from agricultural soils. *Experimental Husbandry* 25:86-95.

Geßler A and Rennenberg H 1998. Atmospheric ammonia: mechanisms of uptake and impacts on N metabolism of plants. *In:* DeKok LJ and Stulen I, editors. *Responses of Plant Metabolism to Air Pollution and Global Change*, pp 81-94. Backhuys Publishers, Leiden.

Gemel J, Waters-Earhart B, Ellersieck MR, Asfaw A, Krause FG, Puri V and Lower WR 1997. Photosynthetic electron transport as a bioassay. *In:* Wang W, Gorsuch JW and Hughes JS, editors. *Plants for Environmental Studies*, pp 209-224. CRC Press, Boca Raton.

Gordon AM, Fisken AJ and Bates TE 1989. Effect of landfill leachate irrigation on red maple *Acer rubrum* L. and sugar maple *Acer saccharum* Marsh. seedling growth and on foliar nutrient concentrations. *Environmental Pollution* 56:327-336.

Grimshaw HM 1989. Analysis of soils. *In:* Allen SE, editor. *Chemical Analysis of Ecological Materials*, 2nd edition. pp. 7-45. Blackwell, Oxford.

Grundon NJ, Robson AD, Lambert MJ and Snowball K 1997. Nutrient deficiency and toxicity symptoms. *In:* Reuter DJ and Robinson JB, editors. *Plant Analysis: an Interpretation Manual*, 2nd edition. Australian Soil and Plant Analysis Council Inc. CSIRO Publishing, Collingwood.

Harper LA and Sharpe RR 1995. Nitrogen dynamics in irrigated corn: soil-plant nitrogen and atmospheric ammonia transport. *Agronomy Journal* 87:669-675.

Harper LA, Sharpe RR, Langdale GW and Giddens JE 1987. Nitrogen cycling in a wheat crop: soil plant and aerial nitrogen transport. *Agronomy Journal* 79:965-973.

Hasselgren K 1992. Soil-plant treatment system. *In:* Christensen TH, Cossu R and Stegmann R, editors. *Landfilling of Waste: Leachate,* pp 361-380. Elsevier Applied Science, New York.

Hasselgren K 1998. Use of municipal waste products in energy forestry: highlights from 15 years of experience. *Biomass and Bioenergy* 15:71-74.

Hernández AJ, Adarve MJ, Gil A and Pastor J 1999. Soil salination from landfill leachates: effects on the macronutrient content and plant growth of four grassland species. *Chemosphere* 38:1693-1711.

Hill RD and Peart MR 1999. Vetiver grass for erosion control, Hong Kong and Guizhou, China. *In:* Wong MH, Wong JWC and Baker AJM, editors. Remediation and management of degraded lands, pp 295-304. Lewis Publishers, Boca Raton.

Hong Kong Observatory (HKO) 2003a. Climate of Hong Kong. Retrieved 23 Sep 2003, from Hong Kong Observatory website: *http://www.hko.gov.hk/wxinfo/climat/climahk.htm*

Hong Kong Observatory (HKO) 2003b. Monthly meteorological normals and extremes for Hong Hong. Retrieved 02 Apr 2004, from Hong Kong Observatory website:

http://www.weather.gov.hk/wxinfo/climat/normals.htm

Hong Kong Observatory (HKO) 2003c. Extract of meteorological observations for Hong Kong. Retrieved 02 Apr 2004, from Hong Kong Observatory website: *http://www.weather.gov.hk/wxinfo/pastwx/extract.htm*

Hopkinson J 2002. The potential of vetiver grass to produce fertile seed when used for roadside stabilisation in Cook Shire. Retrieved 16 Jun 2004, from The Vetiver Network website: *http://www.vetiver.org*

Husted S and Schjoerring JK 1996. Ammonia flux between oilseed rape plants and the atmosphere in response to change in lead temperature, light intensity and air humidity, interactions with leaf conductance and apoplastic NH_4^+ and H^+ concentrations. *Plant Physiology* 112:67-74.

Hutchison GL and Viets FG 1969. Nitrogen enrichment of surface water by adsorption of ammonia volatilized from cattle feedlots. *Science* 166:514-515.

Ingestad T. 198. Relative addition rate and concentration; driving variables used in plant nutrition research. *Plant, Cell and Environment*. 5:443-453.

Jiang B, Wang B, Lan C and Lin L 2000. The purification potentials of different soil with different depths to leachate from Li Keng Landfill in Guangzhou City. *Environmental Science* (in Chinese) 21:32-37.

Jim CY 1996. Edaphic properties and horticultural applications of some common growing media. *Communications in Soil Science and Plant Analysis* 27:2049-2064.

Kapustka L 1994. Terrestrial primary producers. *In:* Calow P, editor. *Handbook of Ecotoxicology*, pp 278-297. Blackwell Scientific Publications, Oxford.

Kapustka LA 1997. Selection of phytotoxicity tests for use in ecological risk assessments. *In:* Wang W, Gorsuch JW and Hughes JS, editors. *Plants for Environmental Studies,* pp 515-548. CRC Press, Boca Raton.

Kemp WM, Caffrey SJ and Mayer M 1990. Ammonium recycling versus denitrification in Chesapeake Bay sediments. *Limnology and Oceanography* 35:1545-1563.

Khan MA and Gulzar S 2003. Light, salinity and temperature effects on the seed germination of perennial grasses. *American Journal of Botany* 90:131-134.

Kimura K, Kikuchi S and Yamasaki S 1999. Accurate root length measurement by image analysis. *Plant and Soil* 216:117-127.

LaBauve JM, Kotuby-Amacher J and Gambrell RP 1988. The effect of soil properties and a synthetic municipal landfill leachate on the retention of Cd, Ni, Pb, and Zn in soil and sediment materials. *Journal Water Pollution Control Federation* 60:379-385.

Landon JR 1991. *Booker Tropical Soil Manual: A Handbook for Soil Survey and Agricultural Land Evaluation in the Tropics and Subtropics*. Longman Scientific & Technical, Harlow.

Lau SP and Fung CH 1999. Reforestation in the countryside of Hong Kong. *In:* Wong MH, Wong JWC and Baker AJM, editors. *Remediation and Management of Degraded Lands*, pp 195-200. Lewis Publishers, Boca Raton.

Legg JO and Meisinger JJ 1982. Soil nitrogen budgets. In: Stevenson FJ, editor. Nitrogen in Agricultural Soils, pp 530-566. American Society of Agronomy, Wisconsin.

Leung CKJ 1985. Phytotoxicity and recycling of landfill leachate [M. Phil. Thesis], The Chinese University of Hong Kong, Hong Kong.

Li XZ and Zhao QL 1999. Inhibition of microbial activity of activated sludge by ammonia in leachate. *Environmental International* 25:961-968.

Liang J, Zhang J and Wong MH 1999. Landfill leachate used as irrigation water on landfill sites during dry seasons. *In:* Wong MH, Wong JWC and Baker AJM, editors. *Remediation and Management of Degraded Lands,* pp 305-317. Lewis Publishers, Boca

Raton.

Lo IMC 1996. Characteristics and treatment of leachate from domestic landfills. *Environment International* 22:433-442.

Lu JCS, Eichenberger B and Stearns RJ 1984. Production and management of leachate from municipal landfills: summary and assessment (EPA-602/284-092). Municipal Environmental Laboratory, Environmental Protection Agency, Cincinnati.

Madejón E, Burgos P, Murillo JM and Cabrera F 2001. Phytotoxicity of organic amendments on activities of select soil enzymes. *Communications in Soil Science and Plant Analysis* 32:2227-2239.

Marschner H 1991. Mechanisms of adaptation of plants to acid soils. *In:* Robert J, Wright RJ, Baligar VC and Murrmann RP, editors. *Plant-Soil Interactions at Low pH*, proceedings of the Second International Symposium on Plant-Soil Interaction at Low pH, 24 - 29 Jun 1990; Beckley, West Virginia, U.S.A. pp 683-702. Kluwer Academic Publishers, Dordrecht.

Marschner H 1995. *Mineral Nutrition of Higher Plants*, 2nd edition. Academic Press, London.

Mass EV 1986. Salt tolerance of plants. *Applied Agricultural Research* 1:12-26.

Mass EV 1993. Plant growth response to salt plants. *In:* Lieth H and Al Masoom AA, editors. *Towards the Rational Use of High Salinity Tolerant Plants,* proceedings of the First ASWAS Conference, 8 - 15 Dec 1990; Al Ain, United Arab Emirates. pp 279-291. Kluwer Academic, Boston.

Matson PA and Vitousek PM 1990. Ecosystem approach to a global nitrous oxide budget. *BioScience* 40:667-672.

McBride RA, Gordon AM and Groenevelt PH 1989. Treatment of landfill leachate by spray irrigation – an overview of research results from Ontario, Canada. I site hydrology. *Bulletin of Environment Contamination and Toxicology* 42:510-517.

McCormac AC, Keefe PD and Darper SR 1990. Automated vigor testing of field vegetables using image analysis. *Seed Science and Technology* 18:103-112.

Menser HA 1981. Irrigation with landfill leachate. *BioCycle* 22:39-41.

Menser HA, Winant WM and Bennett OL 1983. Spray irrigation with landfill leachate. *BioCycle* 24:22-25.

Menzies NW, Skilton JA and Guppy CN 1999. Phosphorus storage on effluent irrigated land. Journal of Environmental Quality 28:750-754.

Mitchel HL and Chandler RF 1939. The nitrogen nutrition and growth of certain deciduous trees of northeastern United States. *Black Rock Forest Bulletin 11*. Harvard University, Cambridge.

Moal JF, Martinez JM, Guiziou F and Coste CM 1995. Ammonia volatilization following surface applied pig and cattle slurry in France. *The Journal of Agricultural Science* 130:183-191.

Murice C, Ettala M and Lagerkvist A 1999. Effects of leachate irrigation on landfill vegetation and subsequent methane emission. *Water Air and Soil Pollution* 113:203-216.

Nadelhoffer KJ 2000. The potential effects of nitrogen deposition on fine root production in forest ecosystems. *New Phytologist* 137:131-139.

National Research Council 1993. *Vetiver Grass: A Thin Line against Erosion.* National Academy of Science, Washington.

Nilsson SI, Berden M, Popovic B 1988. Experimental work related to N deposition, nitrification and soil acidification – a case study. *Environmental Pollution* 54:233-248.

Organisation for Economic Cooperation and Development (OECD) 2000. A comparative review of terrestrial (non-target) plant test methods. Global Crop Protection Federation (GCPF), OECD. Retrieved on 14 Nov 03 from the OECD website: *http://www.oecd.org/dataoecd/41/13/2739855.pdf*

Organization for Economic Cooperation and Development (OECD) 2003. OECD guideline for the testing of chemicals, proposal for updating guideline 208. Retrieved on 02 Sep 2004, from the OECD website: *http://www.oecd.org/dataoecd/11/31/ 33653757.pdf*

O'Brien TA and Barker AV 1996. Evaluation of ammonium and soluble salts on grass sod production in compost. I. Addition of ammonium or nitrate salts. *Communications in Soil Science and Plant Analysis* 27:57-76.

Ottman MJ and Pope NV 2000. Nitrogen fertilizer movement in the soil and influence by nitrogen rate and timing in irrigated wheat. *Soil Science Society of America Journal* 64:1883-1892.

Ozores-Hampton M, Stoffella PJ, Bewick TA, Cantliffe DJ and Obreza TA 1999. Effect of age of cocomposted MSW and biosolids on weed seed germination. *Compost Science and Utilization* 7:51-57.

Pain BF, Phillips VR, Clarkson CR and Klarenbeek JV 1989. Loss of nitrogen through NH_3 volatilisation during and following application of pig or cattle slurry to grassland. *Journal of the Science of Food and Agriculture* 47:1-12.

Pascual JA, Ayuso M, Garcia C and Hernández T 1997. Characterization of urban wastes according to fertility and phytotoxicity parameters. *Waste Management and Research* 15:103-112.

Pastor J, Alía M, Hernández AJ, Urcelay A and Antón FA 1993. Exotoxicological studies on effects of landfill leachates on plants and animal in Central Spain. *The Science of the Total Environment* Suppl. II: 127-134.

Percy I and Truong P 2003. Landfill leachate disposal with irrigated vetiver grass, paper presented at the Third International Vetiver Conference, 6 - 9 Oct 2003; Guangzhou, China.

Pfeffer JT 1992. *Solid Waste Management Engineering.* Prentice Hall, Englewood Cliffs.

Pohland FG and Harper SR 1986. Critical review and summary of leachate and gas production from landfills (EPA/600/2-86/073), U.S. Environmental Protection Agency, Cincinnati.

Qasim SR 1994. *Wastewater Treatment Plants: Planning, Design, and Operation.* Technomic Publishing Co., Lancaster.

Qasim SR and Chiang W 1994. *Sanitary Landfill Leachate: Generation, Control, and Treatment.* Technomic Publishing Co., Lancaster.

Reinhart DR and Townsend TG 1998. *Landfill Bioreactor Design and Operation.* CRC Press, Boca Raton.

Robinson HD and Maris PJ 1983. The treatment of leachates from domestic wastes in landfills-I: Aerobic biological treatment of medium-strength leachate. *Water Research* 17:1537-1548.

Robinson HD 1991. Characterization and treatment of leachates from Hong Kong landfill sites. *Journal of IWEM* 5:326-335.

Rorison IH 1973. The effect of extreme soil acidity on the nutrient uptake and physiology of plants. *In:* Dost H, editor. *Acid Sulphate Soil,* proceedings of International Symposium on acid sulphate soils, 1972; Wageningen, Netherlands. pp 223-253. International Institute of Land Reclamation and Improvement, Wageningen.

Rowell DL 1996. *Soil Science: Methods and Application.* Longman, Singapore.

Rusell EW 1973. *Soil Conditions and Plant Growth.* Longman, London.

Schabenberger O, Tharp BE, Kells JJ and Penner D 1999. Statistical tests for hormesis and effective dosages in herbicide dose response. *Agronomy Journal* 91:713-721.

Schjorring JK 1995. Long-term quantification of ammonia exchange between agricultural cropland and the atmosphere. I. Evaluation of a new method based on passive flux samplers in gradient configuration. *Atmospheric Environment* 29:885-893.

Shrive SC and McBride RA 1995. Physiological responses of red maple saplings to sub-irrigation with and untreated municipal landfill leachate. *Waste Management and Research* 13:219-239.

Skalar 1995a. *The San Plus Flow Analyzer – Water Analysis.* Skalar, Breda.

Skalar 1995b. *The San Plus Flow Analyzer – Soil and Plant Analysis.* Skalar, Breda.

Smith FW and Loneragan JF 1997. Interpretation of plant analysis: concepts and principles. *In:* Reuter DJ and Robinson JB, editors. *Plant Analysis: an Interpretation Manual*, 2nd edition. Australian Soil and Plant Analysis Council Inc. pp 3-33. CSIRO Publishing, Collingwood.

Snyder GH 1996. Nitrogen losses by leaching and runoff: methods and conclusions. *In*: Ahmad N, editor. *Nitrogen Economy in Tropical Soils*, proceedings of the International Symposium on Nitrogen Economy in Tropical Soils; 9-14 Jan 1994; Trinidad and Tobago, pp 427-432. Kluwer Academic Publishers, Dordrecht.

Soil and Plant Analysis Council Inc. 2000. *Soil Analysis: Handbook of Reference Methods.* CRC Press, Boca Raton.

Sommer SG and Christensen BT 1992. Ammonia volatilization after injection of anhydrous ammonia into arable soils of different moisture levels. *Plant and Soil* 142:143-146.

Stebbing AR 1998. A theory for growth hormesis. *Mutation Research* 403:249-258.

Stephens W, Tyrrel SF and Tiberghien JE 2000. Irrigation short rotation coppice with landfill leachate: constraints to productivity due to chloride. *Bioresource Technology* 75:227-229.

Stevenson FJ 1982. Origin and distribution of nitrogen in soils. *In:* Stevenson FJ, editor. *Nitrogen in Agricultural Soils*, pp 22-25. American Society of Agronomy, Wisconsin.

Stevenson FJ and Cole MA 1999. *Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients*, 2nd edition. Wiley, New York.

Sun ST 1987. Fate and efficiency of fertilizer nitrogen (^{15}N labeled urea) applied in wetland rice soil. *Soils* (in Chinese) 19:79-83.

Tam NFY and Tiquia S 1994. Assessing toxicity of spent pig litter using a seed germination technique. *Resources Conservation and Recycling* 11:261-274.

Tamm CO 1991. *Nitrogen in Terrestrial Ecosystems: Questions of Productivity, Vegetational Changes, and Ecosystem Stability* Springer-Verlag, Hong Kong.

Tchobanoglous G, Theisen, H and Vigil SA 1993. *Integrated Solid Waste Management: Engineering Principles and Management Issues*. McGraw-Hill Book Co., Singapore.

Troug E 1930. The determination of readily available phosphorus in soil. *Journal of the American Society of Agronomy* 22:874-882.

Truong PNV 1994. Vetiver grass, its potential in the stabilisation and rehabilitation of degraded saline land. *In:* Squires VR and AT Ayoub. *Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands*, proceedings of the International Workshop on Halophytes for Reclamation of Saline Wastelands and as a Resource for Livestock, Problems and Prospects, 1992; Nairobi, Kenya. pp 293-296.

Tyrrel SF, Leeds-Harrison, PB and Harrison KS 2002. Removal of ammoniacal nitrogen from landfill leachate by irrigation onto vegetated treatment planes. *Water Research* 36:291-299.

US Environmental Protection Agency (USEPA) 1993. Methods for aquatic toxicity identification evaluations: phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity (EPA/600/R-92-080). National Effluent Toxicity Assessment Center, Duluth.

US Environmental Protection Agency (USEPA) 1996. Ecological effects test guideline – Seed germination/root elongation toxicity test (OPPTS 850.4200). U.S. Environmental Protection Agency.

US Environmental Protection Agency (USEPA) 2002. Municipal solid waste in the United States 2000: facts and figures. Retrieved 13 Sep 2003, from the USEPA website: *http://www.epa.gov/epaoswer/non-hw/muncpl/report-00/report-00.pdf*

US Environmental Protection Agency (USEPA) 2003. Municipal solid waste – Basic facts. Retrieved 13 Sep 2003, from the USEPA website: *http://www.epa.gov/epaoswer/non-hw/muncpl/facts.htm*

US Food and Drugs Administration (USFDA) 1987. *Environmental Assessment Technical Assistance Document 4.06*. Centre for Food Safety and Applied Nutrition and Center for Veterinary Medicine, Washington.

Van Dijk HEF and Roelefs JGM 1988. Effects of excessive ammonium deposition on the nutritional status and condition of pine needle. *Physiologia Plantarum* 73:494-501.

Van Ewijk PH and Hoekstra JA 1993. Calculation of the EC50 and its confidence interval when subtoxic stimulus is present. *Ecotoxicology and Environmental Safety*. 1993:25-32.

Vogel F, Hard J, Hug A and Rudolf von Rohr P 2000. The mean oxidation number of carbon (MOC) – A useful concept for describing oxidation processes. *Water Research* 34:2689-2702.

Wang W and Keturi PH 1990.Comparative seed germination tests using ten plant species for toxicity assessment of a metal engraving effluent sample. *Water Air and Soil Pollution* 52:369-376.

Wang W and Williams J 1990. The use of phytotoxicity tests (common duckweed, cabbage, and millet) for determining effluent toxicity. *Environmental Monitoring and Assessment* 14:45-58.

Waskom RM, Westfall DG, Spellman DE and Soltanpour PN 1997. Monitoring nitrogen status of corn with a portable chlorophyll meter. *In:* Hood TM and Jones JB, editors. *Soil and Plant Analysis in Sustainable Agriculture and Environment*, pp 779-794. Marcel Dekker, New York.

Water Supplies Department (Hong Kong) (WSD) 2004. Drinking water quality for the period April 2003 - March 2004. Retrieved 27 Jun 2004, from Water Supplies Department website: *http://www.info.gov.hk/wsd/en/text/water/qualitydata.htm*

Westlake K 1995. Landfill. *In:* Hester RE and Harrison RM, editors. *Waste Treatment and Disposal,* pp 43-68. Royal Society of Chemistry, Cambridge.

Winant WM, Menser HA and Bennett OL 1981. Effects of sanitary landfill leachate on some soil chemical properties. *Journal of Environmental Quality* 10:318-322.

Wong MH and Lau WM 1983. Effects of roadside soil extract on seed germination and root elongation of edible crops. *Environmental Pollution, Series A, Ecological and Biological* 31:203-215.

Wong MH and Leung CK 1989. Landfill leachate as irrigation water for tree and vegetable crops. *Waste Management and Research* 7:311-324.

Wong MH, Li MM, Leung CK and Lan CY 1990. Decontamination of landfill leachate by soil with different textures. *Biomedical and Environmental Sciences* 3:429-442.

Wong RSK 2003. Chemical and ecotoxicological characterization of landfill leachate [M. Phil. Thesis]. The Chinese University of Hong Kong, Hong Kong.

World Bank 1993. *Vetiver Grass: The Hedge against Erosion*, 4th edition. The World Bank, Washington.

Xia HP, Wang QL and Kong GH 1999. Phyto-toxicity of garbage leachates and effectiveness of plant purification for them. *Acta Phytoecologica Sinica.* Vol. 23, digitized periodical retrieved from Wan Fang Data: *http://www.wangfangdata.com.cn*

Xia H, Liu S and Ao H 2000. Study on purification and uptake of vetiver grass to garbage leachate, paper presented at the International Vetiver and the Environment Conference, Jan 2000; Cha Am, Thailand.

Zheng C, Tu C and Chen H 1997. Preliminary study on purification of eutrophic water with vetiver, paper presented at the International Vetiver Grass Technology Workshop, Oct 1997; Fuxhou, China.

Zucconi F, Pera A and De Bertoldi M 1981. Evaluating toxicity of immature compost. *BioCycle* 22:54-57.